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EFFECT OF CHRONIC PAINFUL LESIONS ON DORSAL ROOT REFLEXES IN THE DOG*

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(Received for publication February 14, 1941)

THE CLINICAL observations of Leriche (1939), Livingston (1938, 1940), and others in cases of "pathological" pain syndromes such as causalgia, etc., have been difficult to explain on the basis of accepted concepts of neurophysiology. The persistence of the pain after section of peripheral nerves and dorsal roots and occasional relief for long periods following novocaine injection of an irritative focus or "trigger point" has led one of us (W.K.L.) to postulate that long continued irritation of a sensory nerve might modify the normal physiology of the central nervous system.

The dorsal root reflexes described by Toennies (1938a) might be related to these pain syndromes. The fact that these reflexes are accentuated under certain abnormal conditions, namely decreased body temperature, Barron and Matthews (1939) and Toennies (1939), suggested the possibility of a relationship between dorsal root reflexes and these pain syndromes which are apparently "pathological," *i.e.*, abnormal reactions in certain individuals to irritative lesions.

METHOD

The presence or absence of reflex discharge of fibers in two sensory nerves, the lateral branch of the dorsal radial cutaneous and the saphenous nerves, following single electric shocks applied to the same nerves, was determined in 17 apparently normal dogs. In 8 of these at the time of the first observation two drops of croton oil were injected into the nerve sheath of each nerve. After a period of 21 to 25 days, while the ulcers formed were unhealed and apparently painful, the same nerves on the opposite side were likewise tested for a reflex discharge. In 8 additional dogs with a single painful ulcer of long standing (15 to 40 days) the remaining three nerves were similarly tested. All records were made at normal body temperature of 39.2°C. rectal (no records of spinal temperature were taken). At the conclusion of each experiment observations were made on the effect of lowering the body temperature on the reflexes.†

The animals were lightly anaesthetized with Nembutal, 1 cc. of a 6 per cent solution per 11 kg. body weight. At the level of anaesthesia used the tendon reflexes were always readily obtainable. The nerves were dissected free, cut peripherally and stimulated and led from as indicated in Fig. 1a. A few records were taken with similar leads placed on the dorsal roots.

Monophasic records of both a primary action potential conducted peripherally from the stimulating electrodes as well as the reflex discharge which it produced were obtained. The percentage fiber response was based on the area of the first spike of the reflex discharge compared to the total area of the maximum response of the fibers of fastest conduction (A fibers) as measured from the primary action potential following correction for differences

* Aided by a grant from the Dazian Foundation for Medical research.

† In the first few experiments an ordinary electric heating pad was used to keep the animal's body temperature normal. The alternating current (60 cycle 110 v) induced a current in the animal's body which when led to ground through the nerve was of sufficient strength to excite it. This caused the cord to be conditioned and no reflexes were obtained for as long as 10 sec. after the electrical pad was turned off preparatory to taking records. In the remaining experiments the animals were warmed by a hot water heater.

in amplification necessary to record the two different potentials. A cathode ray oscillograph and differential amplifier, Toennies (1938), was used.

Results

Of the 17 animals with no lesions, 8 showed dorsal root reflexes at normal body temperature and there were no reflexes in 9. Of the 16 animals with lesions, 7 showed reflexes and 9 did not.

In the 8 dogs in which tests were made both before and after lesions, 5 showed reflexes before the lesions and 3 did not. After the lesions, 2 of the animals which had reflexes failed to show them, while 2 of the animals which had previously failed, now gave reflexes.

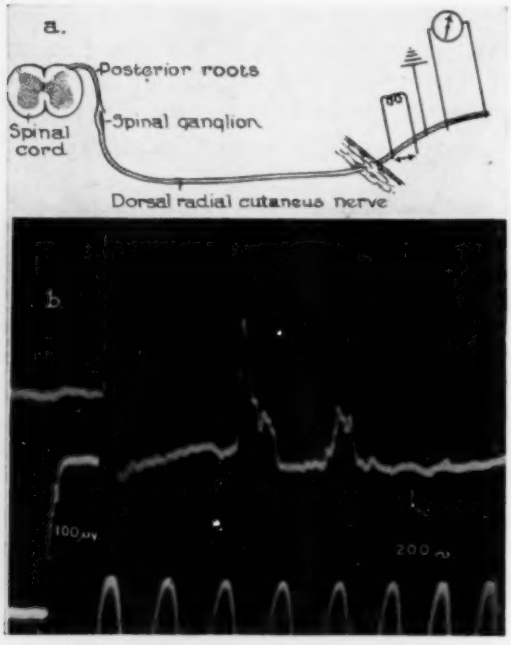

Fig. 1a. Arrangement of the electrodes in stimulating and leading from the nerves.

Fig. 1b. Typical oscillogram. The first spike (incompletely shown because of high gain) is the primary action potential. The reflex response consists of two separate spikes (see text).

The size of the reflexes was no different in the animals with lesions than those without lesions.

In the typical oscillogram, Fig. 1b., the first spike to appear is the action potential in A fibers stimulated electrically and conducted peripherally. The reflex spike appeared 10 to 15 msec. later and in many of the experiments a second reflex spike was seen. It began 18 to 23 msec. following the stimulation. At normal body temperature the first reflex spike usually involved 3 to 5 per cent of the total A fibers. However, in one experiment 9 per cent were estimated to be involved. The reflex was conducted in the dorsal radial cutaneous nerve at 47 to 52 m. per sec. as compared to a rate of conduction of 60 m. per sec. for the fastest fiber in that nerve under the conditions of temperature, etc., for these particular experiments. The second reflex spike, when present, was almost always smaller than the first in area. Its conduction rate, though measured with more difficulty, was approximately the same as the first reflex and did not represent activity in fibers of significantly lower conduction rates such as the reflexes in delta fibers described by Toennies (1938) in the cat.

It was confirmed in the dog, as Barron and Matthews (1939) and Toennies (1939) have shown in the cat, that lowering the body temperature increased the percentage of fibers involved in the reflex or brought a reflex into

view which had not been observed at normal temperature. The optimum body temperature was found to be 36.5°C. Under these conditions reflexes were seen which involved 10 per cent of the total A fibers. In no case in the dog, even on cooling, were reflexes observed which approached the size (65 per cent of A fibers) recorded in the cat by Toennies.

The reflexes were more constant and larger in the dorsal radial cutaneous nerve than in the saphenous nerve. A dorsal root reflex was obtained in every animal except one when the body temperature was lowered.

Conclusion

It appears that the presence of a painful lesion of 15 to 40 days' duration involving a sensory nerve has no influence on the presence or absence of dorsal root reflexes in the dog.

The work of Toennies (1938, 1939) on dorsal root reflexes in the cat was confirmed in the dog. Reflexes were found at normal temperature in about 50 per cent of the animals studied. The reflexes could be produced or increased in size by lowering the body temperature, confirming the results of Barron and Matthews (1939) and Toennies (1939).

* * * * *

We express our appreciation for technical assistance to Mr. Fred Claussen.

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REFLEX ACTIVITY IN THE SPINAL EXTENSORS

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(Received for publication February 15, 1941)

INTRODUCTION

SINCE Adrian and Bronk (1, 2) demonstrated muscle action potentials from single motor units there have been numerous reports of action potentials accompanying artificially stimulated muscle, voluntarily contracted muscle, muscle tension in neuromuscular diseases, and muscle tension occurring in the performance of imaginary tasks (3, 7, 8, 11, 12). In addition, Jacobsen (6) has reported extensive studies of muscle action currents in nervous and "neurotic" patients.

Except for Jacobsen's studies of "neuromuscular hypertonus" and the material which deals with organic neuromuscular diseases, observations of muscle action currents have dealt primarily with artificially stimulated or voluntarily contracted muscle. A few reports indicate an absence of muscle potentials in normal, relaxed muscle. Lindsley (7) stated, "no electrical activity has been demonstrated in any part of a relaxed muscle," on the basis of records, on 6 normal adult subjects, from the deltoid, biceps, flexor digitorum sublimis, branchioradialis, rectus femoris, vastus lateralis, sartorius, gastrocnemius and tibialis anticus. Smith (12) also observed no electrical activity in the relaxed biceps and triceps.

Osteopaths and many orthopedic surgeons report the clinical finding of increased resistance to pressure deformation of the periarticular tissues of spinal, sacro-iliac and appendicular joints in certain cases which manifest neither obvious organic pathology nor voluntary muscle contraction.

In the present study, an attempt has been made, by electromyographic findings, to determine if reflex muscle activity underlies in part these clinical findings.

METHODS

In a majority of instances concentric needle electrodes (22 gauge) were used. Occasionally two plain hypodermic needles, a hypodermic needle and a skin pad electrode, or two skin pad electrodes were used to increase the amount of tissue between leads.

Dual, 4 stage, resistance-coupled, balanced amplifiers drove either loud speakers, a Sanborn cardioscope for visualization, or Westinghouse sensitive bifilar oscillographs for recording on EKG paper. Calibrations were obtained by placing a 10,000 Ω resistor across the leads of one channel and delivering a known input of 10 μV across a 1 Ω resistance to the other (Fig. 1). Both channels were checked by this procedure.

The subject (in a shielded room) was placed prone on an upholstered table, which had a padded slot, 3 in. wide and 6 in. long, for the face. This permitted the head to be in the mid-line position.

Areas of "lesion" and control areas were selected by palpation of the back and electrodes inserted into the erector spinae mass. (Electrodes and skin were treated with 70 per cent alcohol, and the skin was anesthetized by intradermal injection of 1–2 cc. of 2 per cent procaine hydrochloride.) Voluntary contraction, involuntary tension and respiration of the thoracic type are all accompanied by potentials in these muscles (Fig. 2). Hence,

studies of postural or attitudinal reflex activity must be made with the subject completely relaxed and during the few seconds between exhalation and inhalation.

In 6 experiments, after recording from the lesion and normal areas, two additional symmetrical areas, normal on palpation, were studied under comparable conditions.

Subjects

Normal relaxed muscle is soft and resilient; it is not tender to moderate pressure. In the thoracic region the extensor muscle masses can be palpated as columns or segments

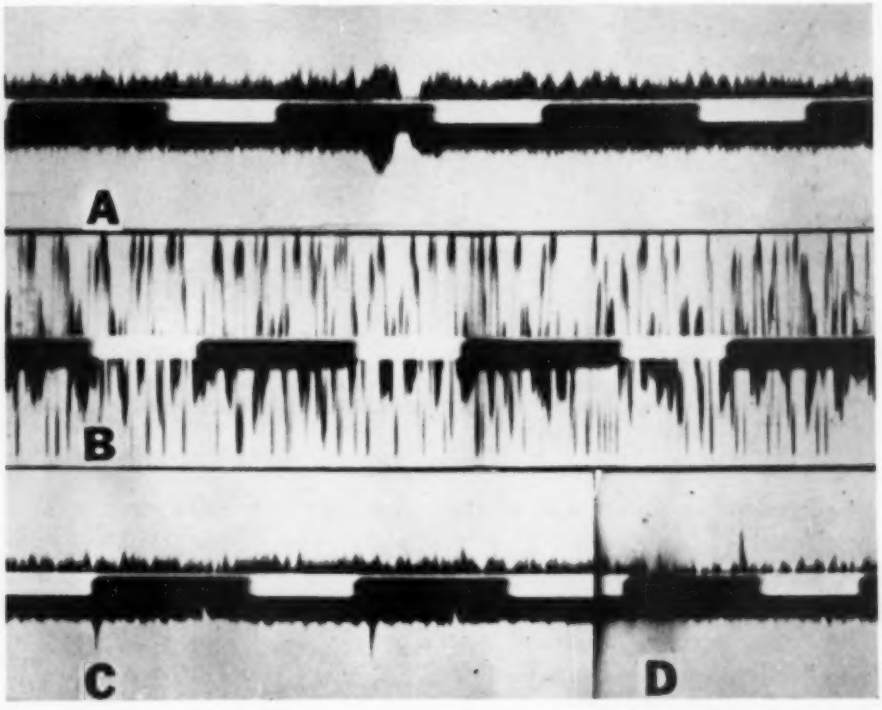

FIG. 1. Upper and lower tracings—concentric electrodes in two normal areas. (A) Subject relaxed at end of exhalation, the single blunt spike is the cardiac artefact. (B) Mild voluntary contraction of the thoracic extensors to demonstrate that both electrodes were in muscle tissue. This control (B) was used in all experiments. (C) Upper tracing—10,000 Ω resistor across input. Lower tracing—input of 10 μV from dry cell and voltage divider. (D) Reverse of (C). Time signal—0.25 sec.

which become firmer on voluntary contraction. Muscle in a "lesion" segment is abnormally firm; it is less resilient and has a characteristic texture which simulates local spasm or contraction even in the relaxed subject. The muscle columns feel indurated and ropy and roll under the fingers. There is abnormal tenderness and deep pressure may cause considerable pain. Such lesion areas are frequently unilateral and limited to one or two spinal segments.

Twenty-five records were taken on 16 students and instructors in whom areas of abnormal tissue resistance were found by palpation. All were free from gross disease, except for one with mild attacks of asthma, and carrying full work. Each one, however, had a postural error in the form of either a slight lateral curvature or an obliteration or an exaggeration of the normal anatomical curves. Some subjects gave a history of having had discomfort in the "lesion" area, others not.

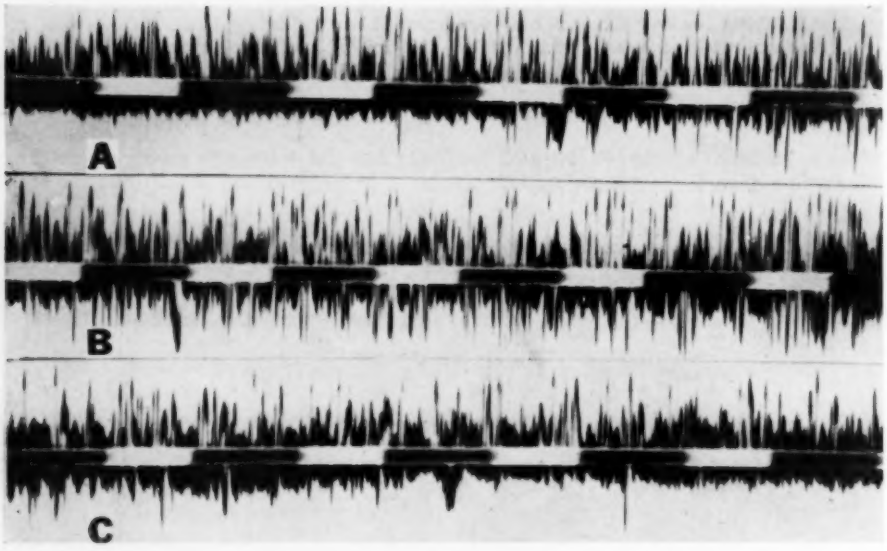

FIG. 2. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Subject starting to inhale with thoracic type of respiration; (B) Subject at height of inhalation; (C) Subject at end of exhalation. Time signal—0.25 sec.

RESULTS

Localized reflex motor unit activity was seen in a lesion area in 21 of the 25 experiments in this series. That this activity was local, and not merely a part of a generalized neuromuscular hypertonus is shown by the absence of activity in an adjacent normal area observed simultaneously.*

The reflex activity recorded varied in degree during each experiment, from occasional quiet periods to activity of single units or of many (Fig. 3). There was no definite pattern to the rise and fall of activity except that the quiet periods were more frequent when the subject first reclined, activity increasing as the subject became fatigued from being in the same position for 30 min. or more. Both the number of active units and the frequency of single units were increased by respiration of the thoracic type, by mild voluntary contraction, and by involuntary "tension."

Single active units were active at frequencies from 3 per second to 24 per sec., but in 23 records, of 29, the frequency was between 6 and 10. While some frequency change occurred without apparent cause (*e.g.* from 10 to 24, B in Fig. 3), the frequency of a single unit was invariably increased by each of the factors listed above.

Inhibition of single units

In individual instances a single active unit in the lesion area could be in-

* In earlier work, with only one channel available, an equivalent observation was made many times by rapid switching over from one area to the other.

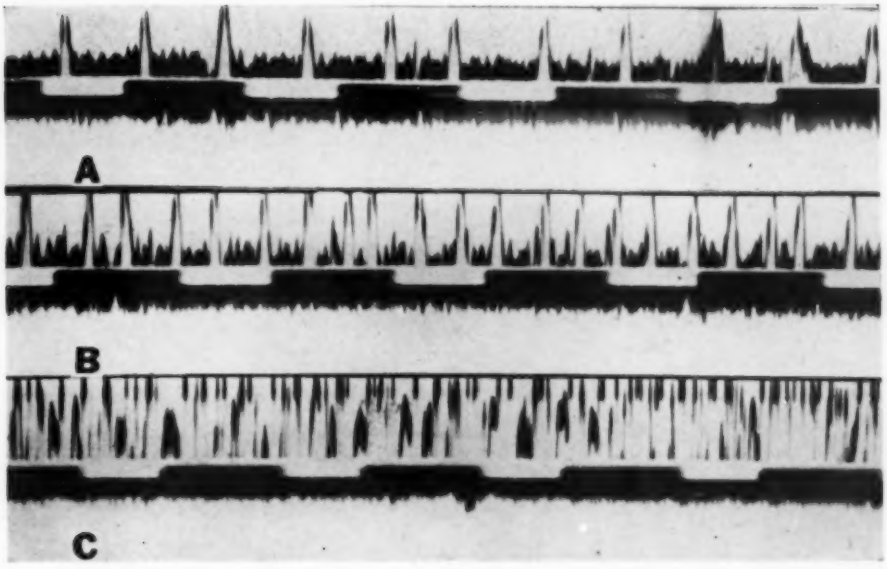

FIG. 3. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Single active unit in lesion area; (B) Either an increase in rate of unit active in (A) or an additional active unit; (C) Numerous units active in lesion area. Time signal—0.25 sec.

hibited by (i) extremely slight voluntary contraction of the abdominal wall, (ii) shallow respiration of the abdominal type, (iii) counting or talking aloud (Fig. 4) and (iv) by swallowing.

Several factors are necessary for the successful inhibition of the reflex activity of a single unit. Most important is the strength or persistence of the active reflex. In some instances this seemed to be "borderline" and the activity came in and faded out. Slight changes in position and momentary periods of voluntary contraction might then result in a cessation of activity. Or, the area might be quiet until activity was initiated by a slight movement of the skin about the electrode.*

Inhibition produced by flexion occurred in these borderline areas, not where the lesion reflex persisted without periods of inactivity.

Additional factors which mitigate against demonstrating flexor inhibition are the presence of more than one or two active units, and the inability of the subject to limit voluntary effort to the flexor groups exclusively but who became tense when trying to follow instructions.

* McKinley stated (10), "As to the movements of electrodes producing action currents, no doubt that happens, and yet we have been able to place our electrodes in the muscles and then massage those muscles in rather lively fashion without any action potentials being developed whatsover."

We have had the same experience. When the electrodes are in normal muscle the skin about them may be moved to a considerable degree without initiating action potentials

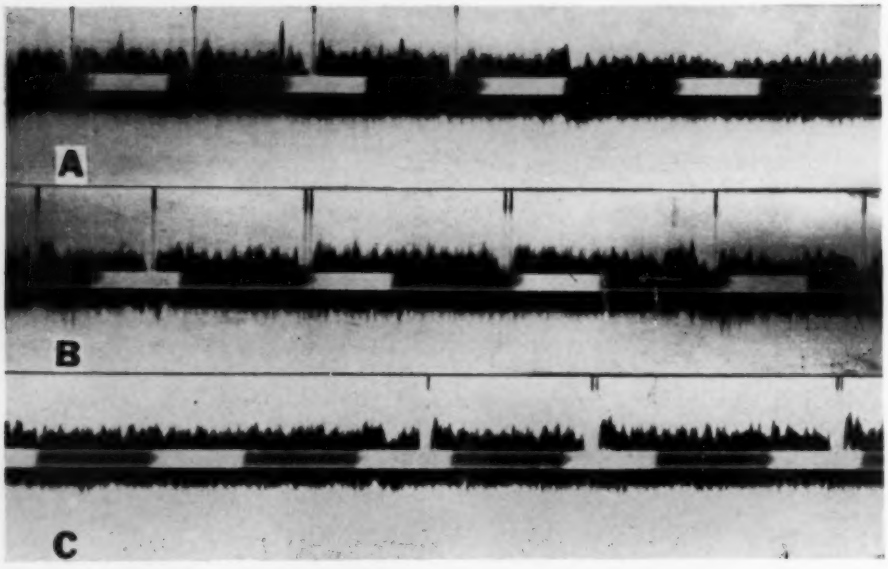

FIG. 4. Upper tracing—concentric electrode in lesion area. Lower tracing—concentric electrode in normal area. (A) Subject inhibited active unit in lesion area by slight contraction of anterior abdominal muscles; (B) Subject completely relaxed; (C) Subject had inhibited active unit by counting aloud, the record shows the unit coming back in. We have been unable to determine if the double spikes in (B) and (C) represent two active units or a peculiar double spike from one. Time signal—0.25 sec.

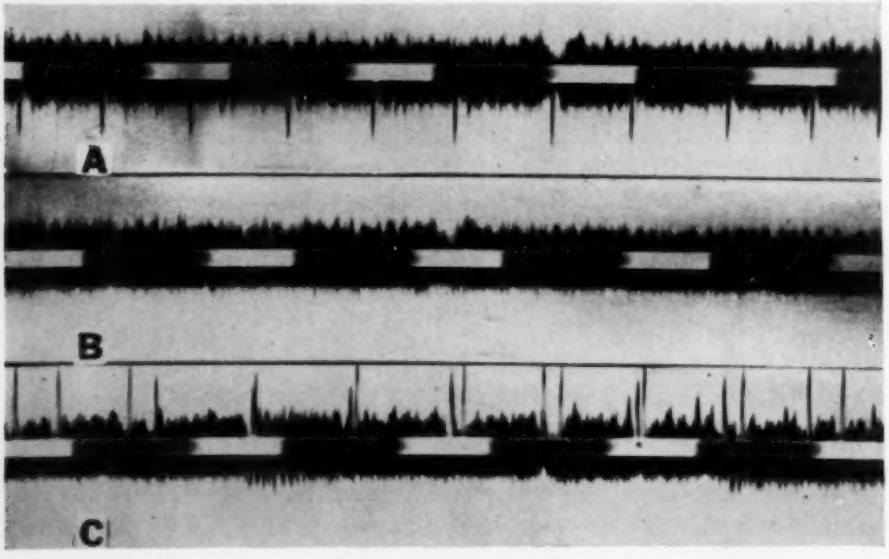

FIG. 5. This record is the first portion of the experiment shown in Fig. 4. The electrode of the upper tracings is in a lesion area, the one in the lower tracings is in a normal area. The active unit of the lesion area had not started to fire when this part of the record was taken. The upper tracing is of the right side, the lower of the left. (A) Subject's head turned toward the left; (B) Head in mid-line position; (C) Head turned toward the right. Time signal—0.25 sec.

Absence of activity in lesion areas

In four experiments no activity was observed in the lesion area. In each of these the observations were made over 30 to 60 min. and the electrodes were slowly removed from the muscle and reinserted at least six times, to insure against missing a small localized area of activity.

Postural reflexes

In the first studies the subject was placed prone on the table with the head turned to one side. Inconstantly, and never in some subjects, varying degrees of activity were seen in the thoracic extensors on the "jaw" side while the "vertex" side was quiet (Fig. 5). Sometimes, when the neck reflexes were not present with the head turned to the degree required by the prone position, activity on the jaw side appeared when the operator forcibly rotated the head farther, or lateral flexed the head toward the shoulder on the jaw side. In other cases forcing the head even to the point of discomfort did not produce activity.

Discussion

The motor unit activity demonstrated in lesion areas is local and is reflex in character. It is similar in frequency, in asynchronism and in recruitment to the stretch reflex as described by Creed, Denny-Brown, Eccles, Liddell and Sherrington (4) with the exception of the finding of low frequency rates in 10 records. There is also similarity with the extensor response in the postural reflex as first described by Magnus (5).

The action potential findings in the lesion area have characteristics identical with the stretch reflex in their response to various tensions and to head position. However, they were observed when the subject was completely relaxed, in the "resting" period of the respiratory cycle and with the head in the mid-line. The activity occurred in local areas where palpation revealed apparent abnormality in the tissue. Hence, they represent a muscle contraction which could be designated as an abnormal reflex.

The difference between the stretch and postural reflexes and the lesion reflex lies in the cause of its development; there being no stretch or positional asymmetry in the latter. The cause is either a stimulation of tension receptors within the muscle itself or stimulation of other receptors which are segmentally related.

Clinical observations have led to a general acceptance of viscerosomatic reflexes, such as the right rectus muscle contraction in cases of acute appendicitis, yet neither biochemical nor bio-electrical studies of these types of "functional pathology" have been made. It is possible that the lesion reflex might be viscerogenic in character. However, this does not seem probable as none of the subjects, with the exception of one asthmatic, was suffering from gross visceral disease.

A more logical explanation is found in the fact that all subjects presented postural abnormalities of various types. As the articular capsules and the

tendon and ligamentous supports of the spinal articulations are richly supplied with proprioceptor end organs, the abnormal pressures and tensions developed in functionally pathologic joints might well cause the lesion reflex. Steindler (13) points out that "under pathologic conditions, however, we may find the spine unprotected against the fluctuations in gravital or dynamic stresses, and strains or even fracture may result." Under these pathological conditions the spinal cord is abnormally bombarded with afferent impulses from the area and a reflex discharge is maintained.

Joints which show some arthritic changes become "stiff" and uncomfortable when the patient reclines for an hour or more. They improve when he moves about. This is probably a parallel to our observation that at times motor unit activity in the lesion area is not seen until the spinal articulations have been relatively immobile for some period of time.

In some experiments slight movement of the skin around the electrode in the lesion area was followed by motor unit activity while the normal control areas, similarly treated, remained quiet. Here the lesion reflex perhaps created an enduring subliminal central excitatory state in a motoneuron pool (5). Additional stimuli from electrode movement, themselves subliminal, can then cause excitation on reaching the motoneuron pool. Conversely, activity of the lesion reflex was temporarily terminated by flexor activity, only when the neurons bombarded from the lesion area were apparently just above the threshold of discharge and could be inhibited by the prepotent flexor reflex.

There are several possible reasons why, in several experiments, no motor unit activity was found in lesion areas. The most likely is that there was none present at that time and that the apparent tenseness in the muscle was due to some factor other than muscle contraction. That activity existed but was not located does not seem likely, as several samplings of the muscle were made by repeated reinsertions of the electrode in each instance. Another possibility is that the presence of the electrodes inhibited activity. Such a phenomenon was surmised by Davis (in discussion of McKinley and Berkwitz, 10) and we have sometimes noted that a lesion area felt softer after the needle was removed. The fact that in some lesion areas there were periods of activity and of inactivity suggest a final possibility that the lesion reflex might be phasic and that when electrical activity was not found it was because the examination was made during a quiet phase.

Summary

1. Reflex muscle activity, similar to stretch or postural reflexes, is seen in areas of "lesion" in the erector spinae muscles when the subject is relaxed, the extermities in symmetrical position, and the head and face in the midline. Adjacent normal areas do not show motor unit activity. One or many motor units may be active, sometimes after a delay of 4 to 45 minutes after inserting the electrodes.

2. Single units discharge at rates of 3 to 24 per second.

3. A single active unit firing intermittently in the lesion area could at times be stopped by flexor contraction.

4. In a few instances motor unit activity was not seen in an area of lesion.

5. In some normal subjects muscle activity appeared in the spinal extensors on the "jaw" side when the head was turned.

We are indebted to Drs. R. W. Gerard and C. Ladd Prosser for their encouragement, suggestions and advice.

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STEADY POTENTIAL FIELDS AND NEURONE ACTIVITY*

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THE ISOLATED FROG BRAIN continues to manifest essentially stationary rhythmic potential oscillations and, under the influence of drugs such as caffeine, slowly traveling potential waves (Libet and Gerard, 1939; Gerard and Libet, 1939, 1940; Gerard, 1941). Such phenomena demand, respectively, the synchronous or successive electrical discharge of large numbers of neurones. Experiments with tiny bits of olfactory bulb, with changed intercellular ionic milieu, with added drugs, with altered temperature, etc., demonstrated that the mechanisms controlling the beat of the individual neurone and the set timing of many neurones are not restricted to the familiar conduction of impulses along neural paths.

The case is especially clear with the large caffeine waves, which travel at about 6 cm. per sec. mainly from the anterior to the posterior pole of the cerebral hemispheres; for these waves are not abolished by nicotine, which blocks synaptic transmission (Libet and Gerard, 1938; Schweitzer and Wright, 1938), and often not even by a sharp complete transection of the entire cerebrum. Since a traveling wave can cross a cut in its path, nerve conduction cannot be responsible; and since a slight separation of the cut halves, even with a film of Ringer connecting them, does block the wave, a chemical mechanism is probably excluded. There remains the possibility of electric currents, flowing through intercellular fluids, as a basis of neurone interaction.

An hypothesis was developed (Gerard and Libet, 1940) which explains the propagation of electric waves along the brain in a manner analogous to the propagation of an impulse along a nerve fiber. The neurones in the frog's hemisphere are in the main oriented alike, with their dendrites directed toward the pial surface and their axones emerging from the ventricular pole of the cell body; and, further, the cell bodies are gathered into a sheet, a few cells thick, paralleling these gross hemisphere surfaces (Fig. 1a. See also Cajal, 1911, p. 844; and Herrick, 1927, Fig. 6 to 9). Assuming that the soma of each neurone is polarized from dendritic to axonic poles (the somatic potential), a D.C. potential maintained by the cell's active metabolism, the whole cell sheet would behave like the polarized membrane of a nerve fiber. A local depolarization, resulting from the discharge of one cell or a few adjacent ones, would permit neighboring cells to discharge through the "leak" and so initiate a spreading wave of depolarization (see Gerard and Libet, 1940, Fig. 16). The "action current," flowing through conducting fluids—

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(*Left*) *A.** Photograph of cross-section through cerebral hemispheres, Rana catesbiana, about 1 mm. caudad to the junction with the olfactory bulbs, stained with toluidine blue for cells. The position of the PV electrodes is indicated. Enlargement 10×.

B. Diagram showing usual position of PV and S_1S_2 electrodes on a longitudinal section of the brain.

FIGURE 1

"horizontally" superficial and deep to the cell layer, and "vertically" between cells and to some extent through the cell bodies—would give the recorded traveling potential wave and would be the means of propagated excitation.

There are some hundred neurones per linear centimeter. To account for the observed rate of travel, a detonator delay (Lorente de Nó, 1935, Eccles, 1936) of 2 msec. at each cell would be required—not an unreasonable figure for cold blooded brain. Also, the caffeine waves tend to an all-or-none character—they usually invade a region suddenly and at full size and maintain their size and rate even when greatly decreased in frequency (Fig. 2A)—although their magnitude can be varied.

Such an interpretation predicts the existence—and direction—of a constant potential gradient between pial and ventricular surfaces; and it makes specific predictions concerning the influence of polarization through or along the hemisphere wall upon the spontaneous waves and those induced by caffeine. These we have investigated.

METHODS

Fluctuating potentials were recorded with Ag-AgCl wick electrodes, 0.2 to 0.8 mm. diameter, shielded nearly to the tip. Two pairs fed independent channels and crystographs, and controls demonstrated the absence of interaction. Polarizing currents were applied also through such electrodes, sometimes with continuing recording of the brain potentials. For measuring D.C. potentials, the more stable Zn-$ZnSO_4$-Agar-wick electrodes were always used, and these sometimes served as well for recording the A.C. potentials simultaneously or for polarizing.

When one electrode was to lead from the ventricle, its wick dipped into the open end of a pipette, filled with Ringer's solution and held horizontally, the narrow (1 mm.) bevelled tip of which was inserted into the ventricle so that the bevel faced the paired electrode on the dorso-lateral pial surface of the hemisphere (Fig. 1). (The pipette tip can be seen

* We are indebted to Dr. O. Sugar for preparation of these sections.

through the thin hemisphere wall, and the pial electrode placed accurately over it.) The ventricle was opened by transecting the hemispheres with a sharp razor near the posterior pole; and the pipette could be inserted to about 2 mm. caudal to the olfactory bulb (Fig. 1b), while the cut medial and lateral walls were gently separated, without its tip producing injury. When the walls were then allowed to collapse, the pipette was "sealed" in place.

D.C. potentials were measured by a balancing potentiometer on a sensitive galvanometer. The high resistance circuit limited current drain from the brain to 10^{-7} A., and this only during the instant that the circuit was closed while attaining balance. Later, even this minimal current was eliminated by using a one-stage high input-impedence D.C. amplifier.* Potentials could be read accurately to 0.01 mV, but the electrode instability was some ten times as great.

Caffeine was applied in 0.5 per cent solution, in which the brain was usually soaked for 0.5 to 2 min.

Steady Potentials

Since the olfactory and other cranial nerves and the medulla are cut in removing the brain, and the hemisphere is sectioned for placing a ventricular electrode, the presence of injury potentials must be considered. Direct measurements, between a freshly cut surface and an uninjured region or the surrounding Ringer's solution film, showed that the initial injury potential of 5 to 10 mV normally falls to less than 1 mV within 10 min. Further, the injury potential in the brain is localized to within 1 mm. of the cut surface, for an electrode placed on the surface 1 mm. from the cut edge is isopotential with one still further away. In the following experiments both electrodes were several millimeters from the injured region and often equidistant from it, so that the potentials recorded were not the result of injury. Further, an electrode in the ventricle commonly showed this to be positive to the outer brain surface. Since any injury produced by inserting the pipette should tend to make the ventricle negative, such a source of error can also be excluded.

The uninjured surface of the cerebrum is not, however, at a uniform potential. Most commonly a regular antero-posterior gradient is present, with the olfactory bulb 0.5 to 2 mV negative to the occipital pole of the hemisphere. A similar voltage gradient in the medio-lateral axis makes the midline up to 1 mV negative to the lateral surface. A fall in temperature decreases these potential differences. As described, both gradients show a progressive, and like, potential rise with increasing electrode separation. There are, however, not infrequent exceptions. Rarely, potential gradients have been encountered of as much as 20 mV on the longitudinal axis and 15 mV on the transverse one. Rather common is the presence of a sharp potential change—as much as 5 mV in 1 mm.—at the junction of olfactory bulb and hemisphere. The bulb may also be positive rather than negative to the hemisphere, and the gradient in the hemisphere may itself be reversed. In fact, the center of positivity or negativity may slowly wander about a single quiescent brain over a period of minutes. (The magnitude of the longitudinal steady potential is not related to the frequency, amplitude or smoothness of spontaneous waves, except that both tend to diminish and disappear together.)

* We are indebted to Mr. G. R. Carlson for building this amplifier and to him and Dr. Helen B. Carlson for assisting in experiments with it.

This lability is distinctly increased by caffeine, the potential between two electrodes changing amount and even direction during a reading. In one case, a reversal and swing of 11 mV occurred within a few min. Rather consistently, this drug diminishes the anteroposterior gradient and often makes the bulb its positive end. These changes may well be related to the potential across the hemisphere wall and are of significance in interpreting the origin of caffeine waves.

According to the hypothesis outlined above, the somatic potentials of the oriented neurones should lead to a potential difference between pial and ventricular surfaces of the hemisphere wall. Such a pial-ventricular potential is observed; we shall call the observed potential the PV potential, in distinction to the hypothecated somatic potential. The PV potential averages 2 mV, P negative to V. It may reach 10 or more or fall to zero or even reverse by one or two millivolts, but large deviations are rare. In any particular brain, the PV potential is fairly stable, ordinarily varying only by tenths of a millivolt from minute to minute. The rhythmic waves are usually abolished when the hemisphere is sectioned, though the PV potential persists.

Caffeine diminishes the PV potential (as the surface ones) to an average of 1 mV in the early stages of its action and to zero in half an hour. The shift, in fact, commonly continues until P is positive to V (up to 3 mV); and this reversal may occur early. The character of the traveling caffeine waves will be seen to be closely related to the PV potential; and their origin near the junction of bulb and hemisphere may well be related to decrease of the somatic potential especially at this region.

Caffeine Waves

Previous records of the caffeine waves had been made from the brain surface, with mono- or bipolar electrode positions. Records taken across the hemisphere thickness, with pial (P) and ventricular (V) electrodes, should eliminate many complications introduced by travel of the wave past successive surface leads, and should more directly measure somatic potential changes. These expectations are borne out. The waves from two surface electrodes (S_1 and S_2) are more complex than those recorded simultaneously from PV electrodes; and the former may change form (presumably as the path of the waves shifts relative to the electrodes and alters the diphasic relations) while the latter remain unchanged (Fig. 7B, see also Fig. 5). The PV amplitude should also be larger than the S_1S_2, by hypothesis; and it is so in fact. (The S_1S_2 wave may be reduced also by diphasic interference.)

A typical PV record of a single caffeine wave is shown in Fig. 2B, and of a train of after-waves in Fig. 2C. The wave starts with a surface positive swing (P+ to V) which passes over to a more enduring surface negative potential. Sometimes a final positive phase appears, although an amplifier artifact may be involved in this. An inconstant surface negative notch may introduce the first main surface positive wave. (Figure 4d shows this in accentuated degree at the arrow. See also 3b, 5f and g.) Commonly superim-

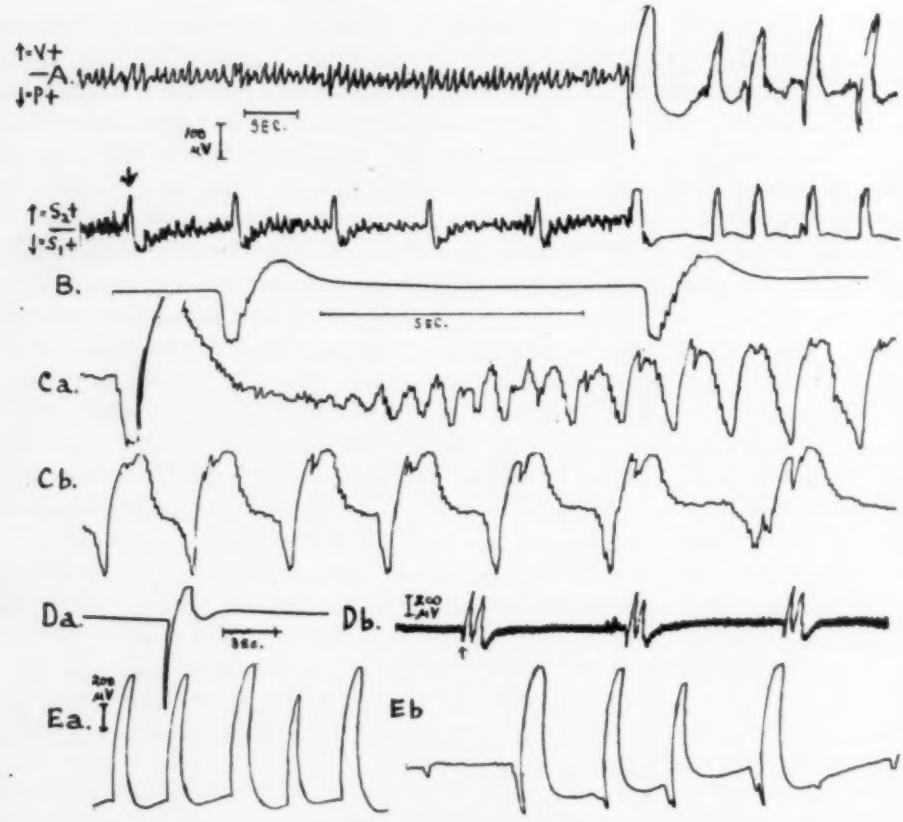

FIG. 2. Caffeine waves. A. Simultaneous PV (top line) and S_1S_2 (bottom line) recording. Caffeinized brain just developing caffeine waves; arrow indicates the first one that appeared. The early waves are originating near S_2, since this electrode first becomes positive and since no activity is then shown by P, near S_1. Each caffeine wave appearing in the PV record starts with P+. Note the sudden reduction of the regular spontaneous rhythm with the onset of the "caffeine waves." (Their presence in the early part of the S_1S_2 record is probably from S_1, which the first caffeine wave does not reach.) Note also that the first caffeine waves appear at full size. The independent records obtained from the two sets of leads are evidence against any electrical interaction between the different pick-ups or amplifying channels.

B. Another brain, caffeine waves, PV recording.

C. Another brain, PV recording. (a) and (b) continuous, except for an interval of 1.7 sec. with one wave.

D. Another brain, (a) caffeine wave, PV recording; (b) during polarization, P+ to V, .01 mA. Arrow indicates surface positive swing; note relatively large size of surface negative waves.

E. Another brain, (a) during polarization, P+ to V, .02 ma.; (b) 10 sec. after cessation of polarization.

A, D, E, are at speed one; B and C at speed three.

Amplification for all is indicated in A, except as otherwise marked.

Note: In this and subsequent figures an up-stroke indicates V+ to P or S_2+ to S_1 (S_1 = the anterior, S_2 the posterior lead on the dorsal hemisphere surface). During polarization shunting by the D.C. current circuit cuts the effective amplification to about $\frac{1}{3}$ the value at other times. The amplification is indicated on records taken during polarization between those electrodes requiring correction.

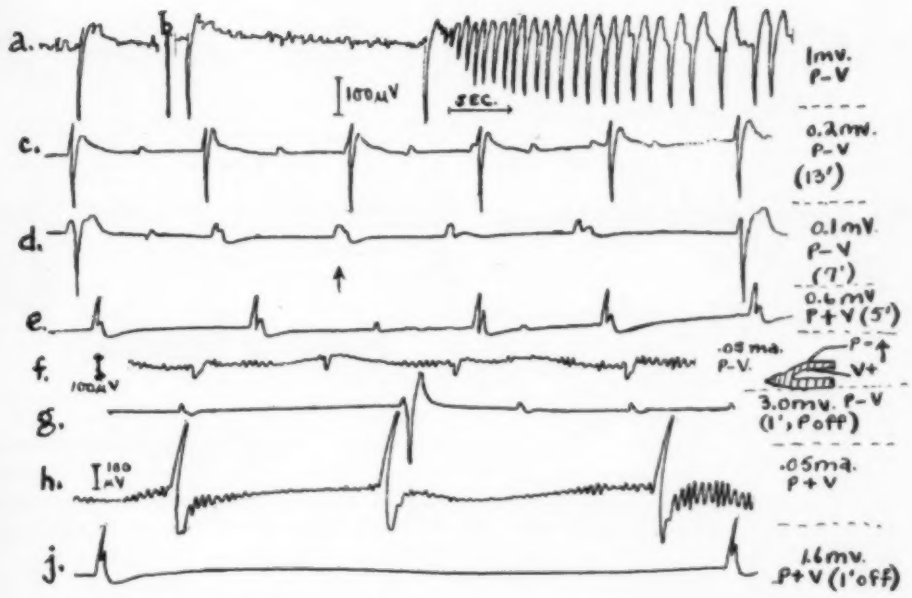

FIG. 3. Caffeine wave changes with D.C. potential (spontaneous and by polarization). PV recording.

a. Caffeine wave at start.
b. 4 sec. after a. Measured D.C. potential (PV) 1 mv. P − to V.
c. 13′ later; D.C. potential 0.2 mv., P − to V.
d. 7′ after c; D.C. potential 0.1 mv. P − to V. (Arrow indicates one of the surface negative waves appearing here among the larger surface positive waves.)
e. 5′ later, D.C. potential 0.6 mv., P + to V.
f. During polarization, P − to V, 0.05 ma. current.
g. 1′ after polarization off; D.C. potential 3.0 mv., P − to V.
h. During polarization, P + to V, 0.05 ma.
j. 1′ after polarization; D.C. potential 1.6 mv., P + to V.

posed upon the main wave are faster components, at frequencies from 40 to 100 per sec., which perhaps are axone spikes distorted in recording (Fig. 2B and C, 4e). (Discharges at a certain level of potential in the slow depolarization waves of neurones are indicated by the results of Adrian, 1931; Heinbecker, 1936; and Barron and Matthews, 1938.) After-waves resemble in form the initial one (*e.g.* Fig. 3a, 4a to g).

The time and potential relations of the main wave, while variable with experimental conditions to be considered, and visually distorted by the arced path of the writing pen, are fairly characteristic. In general, the rising limb of a particular wave is briefer than the following fall. The typical surface positive wave attains about 150 μV and lasts some 130 msec., the negative one is roughly two-thirds this amplitude and essentially twice the duration, although 0.7 sec. may be required for complete return to the base line (Fig. 2B, 4b, 5a). Some voltage loss and distortion of these slow waves are introduced by our amplifiers, of time constant 0.5 sec.

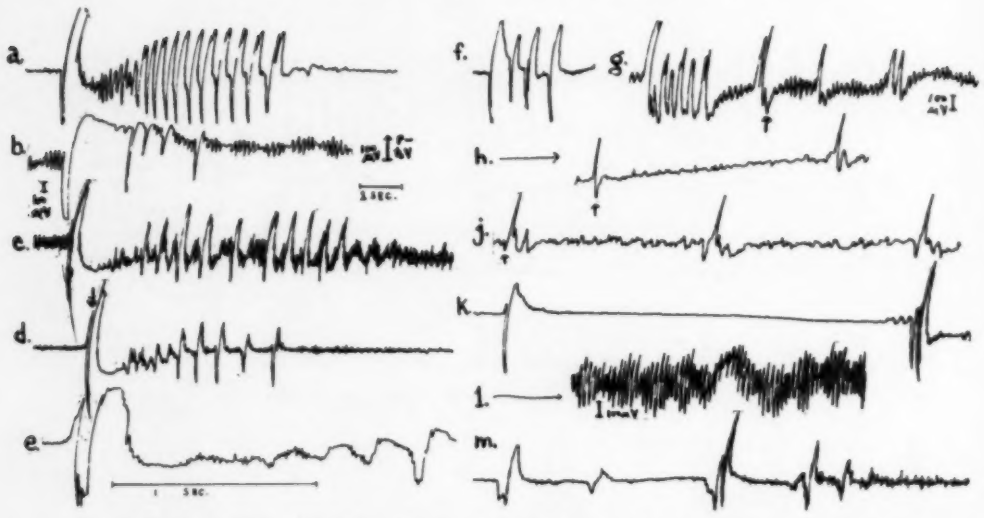

FIG. 4. Effects of pial-ventricular polarization on caffeine waves recorded from same electrodes. All at speed and amplification shown after b, except as otherwise indicated.

a. Caffeine train.
b. During polarization, P − to V, .02 ma.
c. Just after polarization.
d. Same, 2 min. later; arrow indicates an initial surface negative notch.
e. Like d, faster record.
f. 6 min. later.
g. During polarization, P + to V, .05 ma.
h. 5 sec. after polarization off; arrow indicates secondary surface positive wave.
j. Continuation of h (9 sec., without a wave, omitted); arrow indicates initial surface-positive swing.
k. 3 min. later.
l. During polarization, P − to V, .05 ma.
m. 5 sec. after polarization off.

PV POTENTIAL AND CAFFEINE WAVES

If, as hypothecated, the initial surface positive change of a caffeine wave represents depolarization of the existing somatic potential, a close relation should exist between the magnitude and even the direction of the caffeine wave and those of the PV potential. As noted, this latter may spontaneously change, and it is found in fact that the traveling waves alter appropriately. Thus Fig. 3a through e shows a progressive change of the PV potential from the normal, P negative to V, to P positive. The initial positive potential of the simultaneously measured caffeine waves steadily diminishes and finally inverts. (The case of persistent surface positive waves with apparently reversed PV potential, which does occur, will be explained later.) Since the PV potential changes here seen are small and not under control, further studies were made with the aid of constant polarizing currents passed in various directions through the brain.

POLARIZATION

A. Effects on "steady" potentials. A current of 0.05 mA passed for 10 to 30 sec. through the PV electrodes, in contact at their tips, leaves a potential

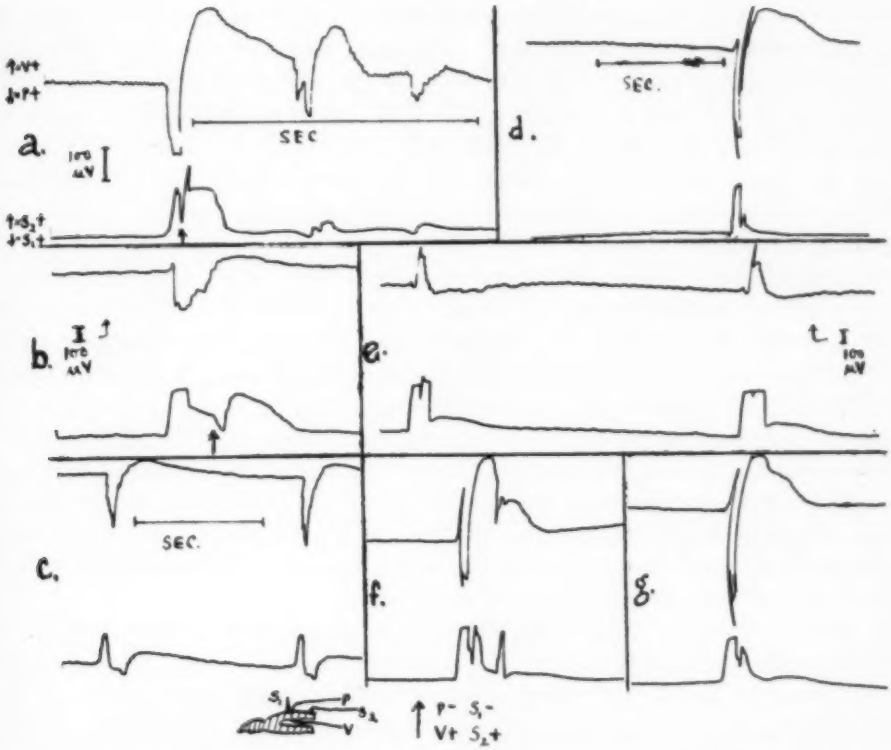

FIG. 5. Effects of pial-ventricular polarization on caffeine waves recorded simultaneously from PV and from surface electrodes. Top line of each pair is PV record, second line S_1S_2.

a. Caffeine wave (amplification is for all records except as indicated).
b. During polarization, P − to V, 0.02 ma.
c. 5 sec. after polarization off. (Slower speed for this and following records.)
d. 1 min. later.
e. During polarization, P + to V, 0.02 ma.
f. 5 sec. after polarization off.
g. 20 sec. later.

difference of about 0.1 mV, in the direction of the applied voltage, which is dissipated within 20 to 30 sec. With the electrodes in position across the hemisphere wall, a similar polarizing current leaves far greater and more enduring potential changes; and the effects have a directional asymmetry.

Thus, 10 sec. after polarization in the direction of the "normal" PV potential (P negative to V) the voltage may be 15 mV. This drops rapidly at first and then more slowly, but even after 6 to 10 min. the potential is 3 to 5 mV and may not return to the original value of 2 mV in another half hour. After a similar polarization in the reverse direction, the immediate voltage may be as great, P now positive to V, but the fall to 3 to 5 mV is achieved in 1 to 4 min.; and by 10 min. the potential has passed through zero to 1 or more mV in the normal direction.

These directional differences are further evidence of a basic polarization —presumably the somatic potential—across the hemisphere wall. That the

retained potential changes after polarization are somehow related to the physiological state (such as the metabolic energy for membrane polarization, the capacity, leakance, etc.) of the cells is evidenced by the altered polarization picture in the caffeinized brain. In this, the post-polarization potential falls rapidly to a steady level, sometimes within one minute and always within five; and this level is essentially the pre-polarization PV potential. Caffeine thus reduces the "polarizability" of neurones as well as their self-generated somatic potential.

Polarizing along the antero-posterior axis also alters the PV potential but to a lesser degree—as would be anticipated from the flow of only part of the current, on the way to deeper tissue regions, perpendicular to the surface. Thus, a current of 0.05 mA passed for 30 sec. from an anterior electrode (S_1) to another (S_2) 6 mm. posterior—S_1 positive to S_2 is a "descending" current—inverts the PV potential measured directly under S_1 from the normal, P = 2 mV negative to V., to P = 1 mV positive. A minute or so later the normal potential is reëstablished. With "ascending" polarization, S_1 negative to S_2, the normal PV potential under S_1 is correspondingly increased. Such local changes in PV potential are found to be causally related to the site of origin of traveling caffeine waves.

B. Effects on waves. When the PV potential is altered during, or even for some time after polarization, in the manner just described, corresponding changes are seen in the traveling caffeine waves. Essentially, as the normal magnitude of the PV potential (pia negative) is reduced, the surface positive phase of the wave diminishes and the following negative phase increases; and when the PV potential actually reverses, so does the sign of the wave. In Fig. 3e, for example, the PV potential had spontaneously become P positive to V and the caffeine waves were accordingly also reversed and starting with a surface negative swing. On polarizing with 0.05 mA, P negative, the normal surface positive wave returned and remained until polarization in the opposite direction again reversed both the PV potential and the wave. (Fig. 3f to j. Note that during polarization the recorded wave amplitude is reduced to about one-third, by the shunting of the polarizing circuit. See legend to Fig. 2.)

The effects of polarization can be followed more intimately when even smaller currents, 0.01 or 0.02 mA, are used. When directed so as to increase the normal PV potential, polarization alters the caffeine wave as follows: (i) the surface positive wave increases in amplitude and (ii) doubles or triples in duration; (iii) the following surface negative wave diminishes in size relative to the preceding swing and often on an absolute basis; (iv) the high-frequency superposed waves are suppressed; (v) the secondary surface positive wave, if present, disappears; (vi) fewer after-wave trains follow the initial complex; and (vii) the velocity of the wave transmission apparently decreases in the region of polarization, as judged from the increased delay of appearance of the surface positive notch at an electrode just adjacent to the region of polarization (see arrows, Fig. 5a and b). These changes slowly

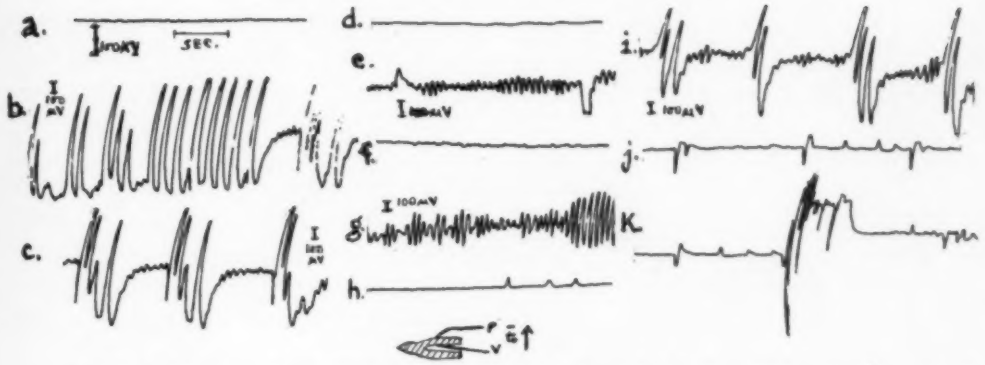

FIG. 6. Initiation of activity in caffeinized brain by polarization. PV recording, except e and f from S_1S_2.

a. Silent caffeinized brain.
b. During polarization, P + to V, 0.02 ma.
c. Same, 1 sec. later.
d. After polarization off.
e. During polarization S_1 + to S_2, 0.04 ma.
f. After polarization off.
g. During polarization, P + to V, 0.02 ma.
h. After polarization off.
i. During polarization, P + to V, 0.02 ma.
j. 5 sec. after polarization off.
k. 3 min. later.

fade during minutes after the polarization is discontinued, except that the amplitude of the initial surface positive wave may sharply fall, to even less than its pre-polarization value (Fig. 5c). (Compare nerve polarization, Bishop and Erlanger, 1926.)

When the polarizing current opposes the "normal" PV potential all these changes occur in the reverse direction; and if the PV potential is sufficiently inverted, the reduced surface positive wave passes over into a surface negative one and is followed by a surface positive wave, which may or may not have been foreshadowed as a secondary surface positivity. With mild polarization, however, the PV potential may be inverted when the waves are not. Polarization may also alter the total incidence of wave trains and single waves, suppress or augment activity; with, commonly, a "rebound" when polarization is stopped. (See Fig. 2D and E; 3f to j; 4, especially b and c; and 5 for examples of these various effects of polarization.)

In Fig. 5 the P electrode (of the PV pair, upper lines) was touching or immediately behind the S_1 electrode (of the surface pair, lower lines). While the PV record is consistent and uncomplicated, the S_1S_2 record can be modified by the position of the electrodes relative to the locus of the wave, the velocity of travel, and the durations of each phase at each electrode. Thus the waves seen in Fig. 5a and in 7B, closely resembling the spike and hump of petit-mal, are to be interpreted as the diphasic result of the appearance of the positive wave at the successive electrodes while the slower negative wave overlaps at both.

The rather regular waves at 8 per sec., seen only during polarization—e.g. Fig. 4b and l—are related to vibration of the electrodes, for they are greatly increased for 2 to 10 sec. after a light tap on the floor or the shielding case. (Such a tap in the absence of polarization causes only a brief flurry of faster waves.) That such waves may not be a simple

mechanical artifact is indicated, however, by the following facts: (i) they are greatly reduced when the same procedure is applied to a dead brain, and change with the condition of the living brain, length of isolation, salt medium, etc.; (ii) they are of a frequency close to that of the spontaneous brain waves, and this fixed frequency is not affected appreciably by wide changes in the pendulum or electrical properties of the electrodes; (iii) they outlast, and are of lower frequency than, the electrode vibration—which can be seen as a separate artifact in the record, lasting only a second or two.

Perhaps the slight oscillatory shift, initiated by the electrode vibration, of the polarizing current among the neurones, acts upon them to enhance and synchronize their spontaneous beats. This point must be further studied; for the present, the waves are discounted as artifacts. (A further, high-frequency thermal-noise artifact complicated records taken during polarization until carbon resistors were replaced with wire wound ones.)

C. Effects on the origin of waves. Appropriate polarization is able to initiate activity in silent brains and to shift the site of origin of traveling waves in active ones. The action of ascending and descending currents on the spontaneous beat of the untreated brain has been described (Gerard and Libet, 1940); and no significant restoration of activity has been observed in the transected uncaffeinized brain, which is regularly silent.

Caffeine waves also are abolished by transection in about one-fourth of the brains, and even without section they gradually "peter out"; and these can often be reinitiated by polarization (see Fig. 6 and 7A). A current of 0.01 to 0.02 mA passed through the hemisphere wall (P positive to V), or one of double strength passed between surface electrodes (S_1 positive to S_2, a descending current), is most effective in initiating activity. In the latter case, waves originate near S_1, equivalent to P positive (Fig. 7A). Stronger PV currents (0.02 to 0.05 mA) are apparently less effective than the weak ones. Polarization with P negative to V occasionally leads to some activity as a rebound after the constant current is discontinued (Fig. 7Ae and f); but usually if any polarizing current is effective the activity induced by it is maximal early in its passage, may decrease during its continued flow, and outlasts its termination by many minutes (Fig. 6 and 7A).

When caffeine waves are present without polarization, their region of origin is shifted, by a current passed between surface electrodes, so as to approach the positive electrode, *i.e.*, S_1 in descending, S_2 in ascending, polarization. (Polarizing P positive to V seems to have a similar but lesser effect, but this point has not been adequately studied.) A descending current also commonly increases the number and frequency of the caffeine waves, and an ascending current has a depressing effect; as was similarly found for the uncaffeinized brain (Gerard and Libet, 1940).

Evidence for the shift in origin of the waves comes largely from the change of wave forms and time relations as recorded simultaneously from PV electrodes and S_1S_2 electrodes, with P at or near S_1. Thus, in Fig. 7Ba, the surface wave starts slightly before the PV one and S_2 first becomes positive to S_1—indicating that the surface positive wave is originating near S_2. A later dip (arrow) with S_1 positive, probably indicates the arrival of the wave at this electrode. In the course of and immediately after polarization (Fig. 7B, c and d), however, the surface wave starts with or just after the PV wave—indicating that the wave is now originating nearer S_1. Later (Fig. 7B,

FIG. 7. A. Initiation of activity in caffeinized brain by polarization. All PV recording at speed shown in a. All records in A and B at amplification shown under Aa, except as otherwise indicated.

a. Inactive caffeinized brain.
b. During polarization along surface, S_1+ to S_2, 0.03 ma. (These large waves decrease during the remaining 30 sec. of polarization.)
c. During polarization, S_1- to S_2, 0.03 ma.
d. After polarization off. Another inactive caffeinized brain.
e. During polarization across pallium, P − to V, 0.02 ma.
f. 5 sec. after polarization off. (No other waves followed those shown.)

B. The influence of cephalo-caudal polarization on caffeine waves. Simultaneous PV (top line of each pair) and S_1S_2 (bottom line) recordings. a and d at speed shown in a; others at speed shown in b. (Note that the PV wave starts definitely after the S_1S_2 wave in a, b, and f, before it in c and d.)

a. Caffeine wave. Arrow points to S_1+ to S_2 notch.
b. Same, faster recording.
c. During surface polarization, S_1+ to S_2, 0.03 ma.
d. 5 sec. after polarization off.
e. 15 sec. after d.
f. 15 sec. after e.

e and f) the original relations are restored. The sign of the surface wave during and immediately after polarization is not simply reversed from that earlier or later; perhaps because of the extra complication of an altered, possibly inverted, PV potential due to the polarization and of changed diphasic interference.

Shifts of the locus of origin of the traveling waves, aside from any effects of polarization, also make uncertain the determination of changes in their velocity. Actually the situation in the whole isolated brain may be quite complex, with the main wave traveling from the olfactory bulb to the occipital pole and with secondary and satellite waves "firing back" from the latter. This is indicated by the times of appearance of the various waves at two sets of paired surface electrodes, and by the fact that cutting away the occipital pole regularly changes a polyphasic main wave in the anterior portion to the typical simple diphasic one and eliminates entirely the following satellite waves.

Discussion

The new observations here reported were mainly suggested by the hypothesis outlined, and they support it as a whole. We do not claim, however, that all facts are accounted for nor that the present crude picture will prove adequate. The spontaneous reversal of the PV potential, without wave inversion, is especially disturbing. On the other hand, the existence of a somatic potential, and the consequent electric fields playing upon masses of neurones, would offer an explanation of many other puzzling neural phenomena.

It has been pointed out elsewhere (Gerard, 1941) how the mass functioning of the cortex in learning (Lashley, 1929) and of the neuraxis in development (Coghill, 1940) or regrowth (Weiss, 1936), the establishment of new functional neural connections between brain centers in conditioning, the existence of synchronized action of many neurones in the retina (Adrian and Matthews, 1928) or brain (*e.g.*, Jasper, 1937) or of other cells not in physical contact (*Nitella*, Hill, 1939), the decrease of neural metabolism associated with central inhibition (Dann and Gardner, 1930), the summation of excitatory and inhibitory impulses reaching separate neurone processes (Gerard, 1932), etc., are accounted for in terms of intercellular potentials and currents more satisfactorily than in terms of nerve impulses conducted along anatomically set pathways.

Further, much evidence is accumulating to show that action currents in nerve fibers can influence the threshold of the same fiber beyond a block (Hodgkin, 1937; Lorente de Nó, 1939) or of a separate but nearby fiber (Katz and Schmitt, 1940; Blair and Erlanger, 1940), or can even excite responses under these conditions (Erlanger and Blair, 1940; Tasaki, 1939; Jasper and Monnier, 1938; Feng and Li, 1940).

There are good physical reasons for expecting more marked effects in the case of cell bodies. These are regularly free of myelin and so more accessible to intercellular currents; and their considerably greater size would expose them to a greater voltage drop in a given potential field than would be the case for a fiber. Also, as a current generator, the more separated poles of a somatic potential, as compared to that across a nerve membrane, would cause a greater current flow at a distance from the battery in the volume conductor. Offner and Winternitz (unpublished) have found, for the inverse case of a pick-up electrode, that the fraction of a fixed potential picked up in

a volume conductor decreases as the square of the distance from the source with a monopolar electrode, as the cube with a concentric one. And the finding that the caffeine wave crosses a complete anatomical section permits only an electrical explanation of some sort.

The measured PV potential is not proof of the existence of the postulated somatic potential. The former might be due to potential gradients in other structures than neurone somas (as ependyma, glia cells, nerve fibers, etc.), or to potentials between cell layers rather than within cell units. Experiments in progress with microelectrodes should make the interpretation more certain;* but if the measured potential is in fact that along the neurones' axes, many of the observed phenomena receive an easy explanation, and we shall assume that such is the case. Many details of the caffeine waves, however, are not simply accounted for on these lines and it is not anticipated that the present interpretation will remain adequate without later accounting for further complexities which are at present neglected.

If the main surface positive wave represents a discharge of the somatic potential, for example, what is the basis for the following negative wave, or the occasional preceding negative notch (Fig. 3c, 4d, 5f and g), or the secondary positive wave (Fig. 4g and h, see arrows), or the absence of the main wave with the presence of only the negative variation which normally follows (Fig. 3e and h, 4g and h). The regular negative wave behaves in general as if it were an after-potential, yet what seems to be this wave can be present with the preceding positive wave appearing or disappearing (*e.g.* Fig. 4h to j). Another type of negative wave seems unrelated to any positive potential and may appear as an alternate to it (Fig. 3c, d, and possibly e). This wave does not travel (the other nearly always does), but is encountered simultaneously—within 10 msec.—at electrodes widely spaced on the pallium.

Whether these latter potentials are due to other units than those responsible for the ordinary moving waves, or whether the same units can manifest potentials of opposite sign (as claimed for the retina, Granit and Helme, 1939, and for sympathetic ganglia, Eccles, 1936, see also 1939), we cannot say. On the first interpretation, deep units becoming active, and so negative as usually pictured, would probably account for the surface-positive wave, superficial units for the negative one. There is evidence that the positive and negative waves obtained from the mammalian cortex by electrical (Adrian, 1936) or strychnine (Dusser de Barenne and McCulloch, 1938) stimulation do depend on such separate cell layers. The results on frogs, however, do not fall into such a picture. For example, when a brain is placed in caffeine it would be anticipated that the superficial layers would be earliest affected and the surface-negative wave appear first—as is the case with strychnine in the cat—but this does not happen (*e.g.* Fig. 2A). Also, polarizing P positive to V should, if anything, differentially depress the superficial layer in the anodal region, yet it increases the negative wave. Nor is there

* In experiments to date, carried out by Alene Silver, no sudden voltage changes have been encountered at the depth of the layers of cell bodies.

any explanation, in terms of layers, of the reversal of sign of the caffeine waves with that of the PV potential.

Some further evidence bears upon two related problems; of the initial surface negative notch (which can occur even when P is normally negative to V—Fig. 3a, c, d, and g), and of the occasional presence of caffeine waves of normal sign with an inverted PV potential, or vice versa (Fig. 3c, d see arrow, and g). When the PV potential is followed continuously, it has been observed that a relatively steady potential, P positive to V, may swing rapidly to the normal direction (P negative), hold the new value for some seconds, and then return to the original conditions. Such shifts last about as long as a single train of caffeine waves and are repeated at the intervals at which these trains occur in the same brains. Whatever such PV shifts may mean, they seem rapid enough to give the initial negative notch on the "leader" wave (to which this notch is limited), and they could account for cases of apparent conflict between the signs of the PV potential and the traveling waves.

A final point may be made on the influence of polarization upon the caffeine waves and the PV potential. During the flow of applied current both alter together as expected, but when the current is stopped the steady potential falls off progressively towards the initial state while the waves often show a prompt "rebound" in the opposite direction (see also Gerard and Libet, 1940). Clearly other factors than the magnitude of the PV potential are involved, a change in cell irritability—as in polarized nerve—comes to mind.

Despite such additional problems, however, it is established that steady potentials do exist among brain cells and significantly influence their behavior. When these potential gradients alter, spontaneously or under the influence of applied currents, there occur changes in the oscillating potential waves—both traveling and stationary—which can be rationalized by the interpretations here developed. These changes include: their appearance or disappearance; a shift in their site of origin; their magnitude and sign; etc.

The probable importance of intercellular currents in synchronizing the action of many neurones has been emphasized earlier (Gerard, Marshall and Saul, 1936; Gerard, 1937; Libet and Gerard, 1939), and their role in producing traveling waves is here elaborated. The phenomena of electronarcosis (Silver, 1939) and electro-"epilepsy" are probably related. The focal origin of true epileptic seizures and their progressive spread do strongly resemble the phenomena in the caffeinized frog brain. The failure of waves, induced by strychinine (Dusser de Barenne and McCulloch, 1937) or accompanying epilepsy (Erickson, 1940), to travel across a cut in the mammalian cortex may indicate different mechanisms in these cases or may result from the relatively poor apposition of the cut surfaces achieved in a large brain *in situ*, subject to pulsations and bleeding at the cut.

What structural and metabolic machinery within a cell keeps charging the somatic potential battery despite the intercellular current leakage; and

what the relation is between the somatic potential and that recorded between different surface points of the neuraxis (see also Burr and Harman, 1939, who find the rat hemisphere as much as 16 mV positive to the sciatic; and Burge, Koons, and Burge, 1940) are questions for the future. Similarly, for the mechanism by which caffeine and like agents evoke traveling waves; although the evidence of decreased polarizability and steady potentials in the caffeinized brain suggest an action on membrane impedance (see Spiegel and Spiegel, 1939).

Finally, the somatic potential may supply the needed factor of integration for activity of a single neurone. The problem of how impulses reaching different dendrites of a cell can sum their influences, and the more difficult one of the summation of excitation and inhibition, have concerned many workers. If all synaptic potentials contribute to a change in the overall somatic potential, to alter it in one direction for excitation and in the other for inhibition, these difficulties are overcome (Gerard, 1941). There is indeed evidence: that nerve cells can manifest large spontaneous potentials over cell dimensions, and evoked potentials can be of either sign, depending upon where incoming impulses reach the cells (Renshaw *et al.*, 1940); that a change in potential of the axonic poles is related to the level of cell activity (Lorente de Nó, 1939); and that an anatomically polar distribution of particular synapses exists for the Mauthner cell (Bodian, 1937).

Summary

The continued travel, after neural connections are blocked or severed, of potential waves along the hemisphere of the isolated frog brain, depends upon the existence of intercellular currents. These are assumed to result from the existence, and discharge, of a potential, the somatic potential, from axonic to dendritic pole of the similarly oriented neurones. Such a polarized sheet of cells should behave formally like a polarized nerve membrane; and a number of predictions from the hypothesis have been tested and confirmed.

Steady potentials, relatively constant in time, have been observed between various surface points and, especially, between the uninjured pial and ventricular surfaces of the hemisphere wall. The PV potential is normally 2 to 3 mV, with P negative to V, but may slowly change magnitude and even direction, especially under the influence of caffeine.

The traveling wave induced by caffeine is simpler and of greater amplitude as recorded from PV electrodes than from surface ones. Its characteristics are described; essentially, a large surface positive wave is followed by a feebler and longer surface negative phase.

Polarization through the thickness of the pallium, by as little as 0.01 mA for a few seconds, changes the PV potential in either desired direction. The new potential returns to the initial value during 10 to 30 min. after the applied current is ended, but shows an asymmetry with the direction of polarization. The caffeinized brain is less "polarizable."

As the PV potential is altered, spontaneously or by an applied current,

the caffeine wave alters with it; and the two commonly reverse sign together. Some but not all exceptions are explained in the text.

"Depolarizing" the PV potential at some position on the hemisphere can initiate caffeine waves in a previously quiescent brain or can shift the source of waves already present so that they originate at this locus.

These findings support the hypothesis advanced. Some limitations and extensions of an interpretation of neurone interaction in terms of electric currents are considered.

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FATIGUE AND REFRACTORINESS IN NERVE

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IN A PRELIMINARY COMMUNICATION (Brücke, Forbes, and Early, 1940) we reported that prolonged repetitive stimulation of medullated nerve delays the recovery of responsiveness (size of maximal response) in the relative refractory phase, and to a much greater extent delays recovery of excitability. The present report amplifies our observations on these points and brings out some further information on fatigue and refractoriness.

METHOD

Single shocks used for conditioning and testing stimuli were delivered by Harvard coils to excised frog sciatic nerve as described in our prevous paper (1941). Measured control of the strength of the testing stimuli was obtained by varying the resistance in the primary circuits. In experiments in which prolonged activity of the nerve was required, a photo-electric cell provided the fatiguing stimuli, generally at a frequency of 240 per sec. In later experiments the fatiguing shocks were delivered through a one-to-one transformer to assure the avoidance of direct-current components. The fatiguing stimulation was interrupted by the opening of the first key of a Lucas pendulum, the opening of the second or third key inducing the testing shock. Usually the interval between the cessation of the fatiguing stimuli and the test was 80 msec. Further tests could not be made at intervals shorter than 2.5 sec. Testing and fatiguing shocks were always sent through the nerve in the descending direction through two separate pairs of Ag electrodes (see inset Fig. 4). The action potentials were led through a Grass condenser-coupled amplifier and registered on a cathode ray oscillograph adjusted for a single sweep which was controlled by the Lucas pendulum to start simultaneously with the conditioning shock or somewhat earlier.

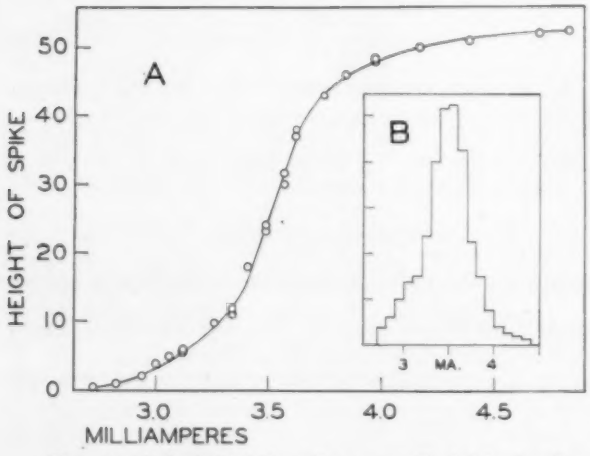

FIG. 1. A, height-intensity curve (height of spike against strength of stimulus) of resting sciatic nerve. B, distribution of α fibers according to thresholds, derived from A. (Frog, 11/6/40. Temp. 23°C.)

In a many-fibered nerve excitability cannot be measured directly, as is possible, for instance, in a single fiber or in the heart. The heights of the responses cannot be used of themselves as an indication of excitability because there is no proportionality between height of response and strength of stimulus. In this paper calculation was generally made by the method of Graham and Lorente de Nó (1938) from the height of submaximal spikes according to their position on a height-intensity curve which was taken immediately before the fatiguing series or as soon as the nerve had completely recovered. In this way "it was possible to determine the strength of shock to which the constant testing stimulus had been made equivalent by the conditioning process" (*loc. cit.*, p. 327). The ratio of the actual strength of the test shock to the strength to which it became equivalent after fatigue provides a quantitative percentile measure of the decrease of excitability. If, for instance, the actual strength of the stimulus used was 5 mA. and the strength to which it had be-

come equivalent after fatigue was only 4 mA., we may say that excitability had decreased to 80 per cent. In this case an increase of the actual stimulus by 25 per cent to 6.25 mA. would compensate for the decrease of excitability, that is to say, it would bring back the least excitable fibers that had dropped out after excitability had decreased.

With regard to this indirect method of calculating excitability it might be of interest to consider that the smoothness of the height-intensity curve of the α fibers in a nerve is not self-evident. The distribution of fibers in the saphenous nerve according to diameter is so irregular (cf. Gasser and Grundfest, 1939) that thresholds of the fibers in a mixed nerve—generally linked to size—might be expected to be distributed irregularly also. This is not the case. Figure 1A shows a height-intensity curve taken from a resting nerve. In B the differences in height of responses to stimuli of increasing strength are plotted against stimulus strength. This curve (B) represents the distribution of α fibers according to their thresholds. We must bear in mind, however, that it is somewhat distorted because the height of the spike does not change in strict proportion to the increase in number of fibers responding. Except for a slight asymmetry this curve corresponds sufficiently well to a normal curve of distribution. In a few experiments the above method was compared with the classical scheme of varying the strength of the stimulus until the response was the same as that obtained before excitability had changed. Although both methods gave similar results, finer changes in excitability were detected by the classical procedure (direct method).

Results

To avoid confusion between the lasting effects of prolonged stimulation and the transient refractory phase following a single response, we shall speak of the former as "fatigue" and shall use the word "conditioned" to denote the effect of a *single* preceding impulse. The word "resting," when used, signifies unconditioned, without reference to recent prolonged stimulation.

Recovery of excitability after prolonged activity. The combined effects of prolonged activity and refractoriness may be studied by recording revovery of the spike potential (i) at a constant interval after a conditioning shock at different times after the end of the fatiguing stimulation or (ii) at a fixed time after the end of the tetanization with a variable interval between the conditioning and the testing stimuli. The first of these methods was almost invariably employed; the curves in Fig. 11, however, were obtained in the second way.

Figure 2 is the photographic record of the recovery of a conditioned submaximal response after fatiguing stimulation, the interval between the conditioning and the testing stimuli being held constant. When in successive experiments an unconditioned submaximal spike potential and a conditioned submaximal spike potential were reduced by fatigue to the same height, they were both found to follow similar courses of recovery; but a thorough investigation of this point was not made.

In another group of experiments we determined the strength of stimulus required to evoke a submaximal unconditioned response of a fixed height. The strength of stimulus was then increased until a conditioned response of the same height was obtained. These two stimuli, the stronger for the conditioned response, the weaker for the unconditioned, were used to test the reduction of excitability after fatiguing tetanizations of a given length. It was found that excitability was reduced much more in the resting (unconditioned than in the refractory (conditioned) nerve. Reduction of excitability in the

latter case depended upon the interval between the testing and the conditioning shocks; the longer this interval (that is, the weaker the testing shock required), the greater was the depression due to the fatiguing tetanization. An experiment of this kind is represented in Fig. 3. Excitability was reduced by fatigue to about 96 per cent of normal when the nerve was tested with strong shocks 2 and 3.2 msec. after the conditioning shocks, and to about 88 per cent when tested with a weaker shock later during the refractory state.

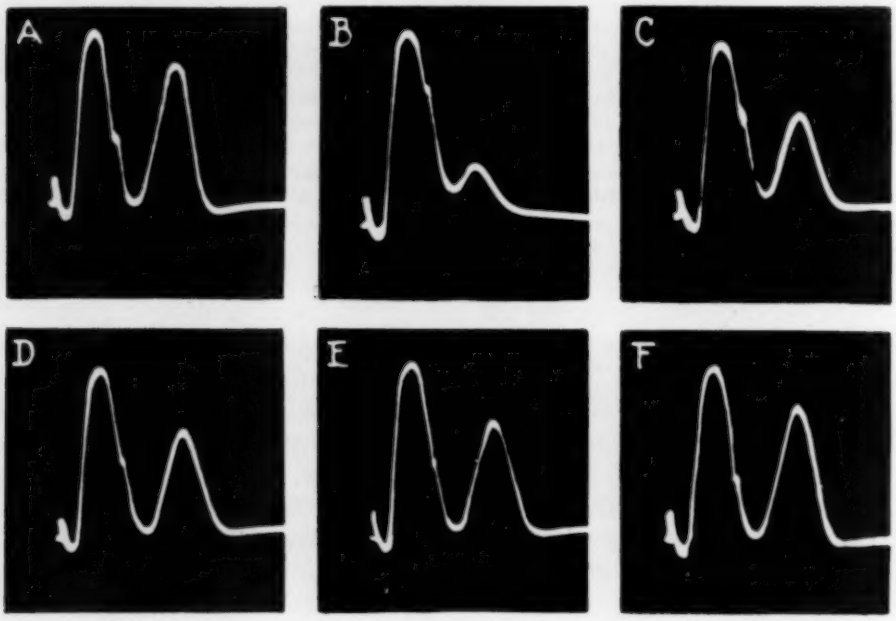

FIG. 2. Recovery of a conditioned spike potential with testing shock submaximal. Interval between conditioning and testing shocks 1.5 msec. A, response in unfatigued nerve, pictures B to F taken 40 msec., 6, 26, 80 and 140 sec. after the end of a 15 sec. tetanization (120 per sec.). The shifting of the artefact of the second induction shock along the descending part of the first spike potential indicates the increased duration of this spike after tetanization. (Frog sciatic nerve.)

When the unconditioned response to a *single* testing shock was observed, however, excitability was found to have dropped to from 80 to 83 per cent.

It became evident that there was need of investigation of the effects of prolonged activity on excitability in nerve in the resting state. Hence, experiments were performed which gave the following results. Rate of recovery of excitability depends directly on the duration of the fatiguing stimulation This is shown in Fig. 4, where recovery of excitability to 95 per cent takes place at 16, 20 and about 100 sec. after tetanizations of 3, 5 and 15 sec. respectively. These results recall the fact that the "second" positive after-potential increases in duration as well as in size as the length and the frequency of the tetanus are increased (Gasser, 1939). According to Gasser and Grundfest (1936) the positive after-potential in a warm-blooded nerve was

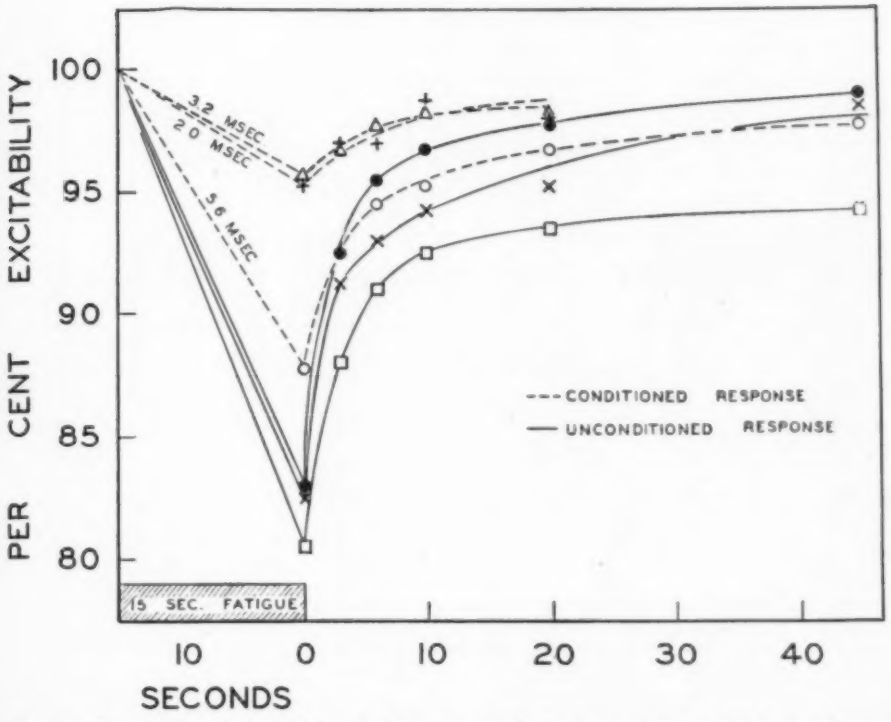

FIG. 3. Recovery of excitability after 15 sec. fatiguing stimulation. In each experiment the testing shock before fatigue provoked a submaximal response of identical height on the screen.

Dots, squares and x's, using single testing shocks (10:16, 10:33 and 11:02). Intensity in primary circuit: 9.6, 10 and 10 mA.

Crosses, triangles and circles, using conditioned stimuli after intervals of 2.0, 3.2 and 5.6 msec. Intensity in primary circuit: 67, 30 and 13.5 mA.

(Frog sciatic nerve, 10/7/40. Temp. 23.8°C.)

visible for 1 to 2 min. after a 10-sec. tetanization and for more than 4 min. after a 30-min. tetanization.

Experiments such as the one represented by the curves in Fig. 5 show that fatigability does not vary in different α fibers of a given nerve. These curves are height-intensity curves of one nerve taken (i) in the unfatigued state, (ii) after 5 sec. and (iii) after 10 sec. fatigue. The relative excitability in the fatigued nerve was calculated for five different heights of response by comparing the strength of stimulus (mA.) necessary to obtain each of these responses with the stimulus required for an equal response in the resting nerve. At all the five points considered, excitability was reduced by the two fatiguing periods in approximately the same relation (100:84:80). Since the ratio of the strengths of stimuli necessary before and after fatigue to obtain a certain height of response is a measure of the *loss* of excitability, and this loss is found to be the same at all points along the height-intensity curve after a fatigue of a given duration, the effect of fatigue is concluded to be the same for all α fibers of a given nerve.

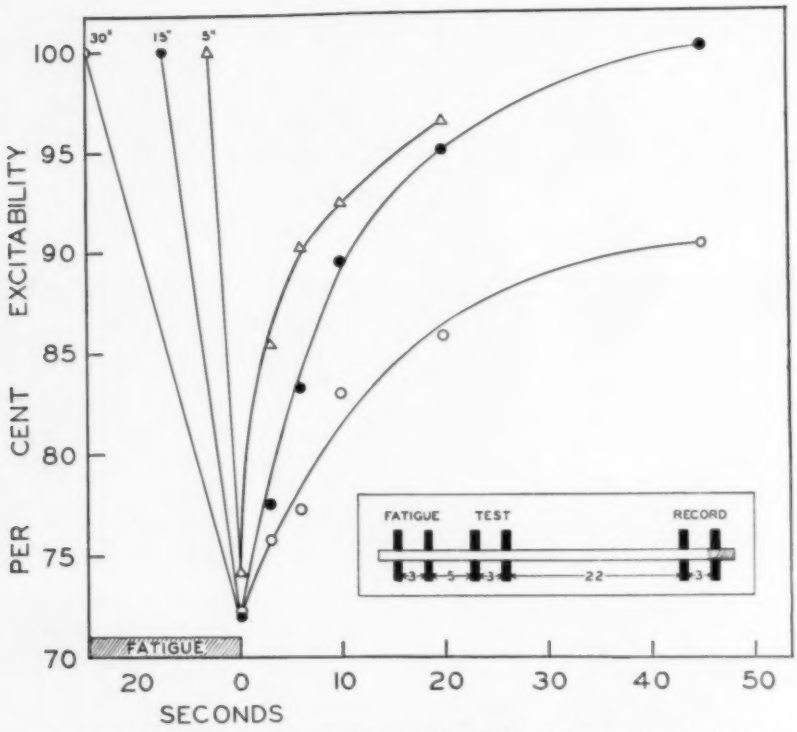

FIG. 4. Recovery of excitability after fatiguing tetani of 5, 15 and 30 sec. durations. (Frog sciatic nerve, 10/7/40. Temp. 23.8°C.)
Inset: Arrangement of nerve on electrodes; dimensions in mm.

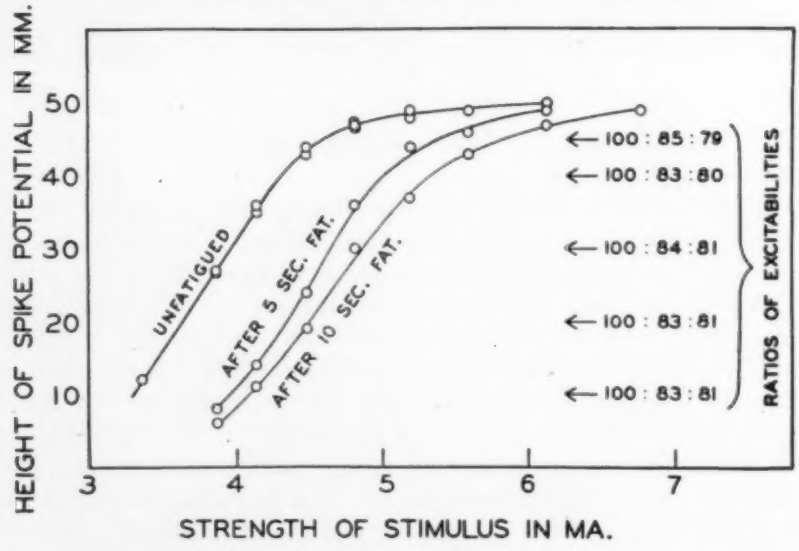

FIG. 5. Three height-intensity curves showing the same decrease in excitability in different groups of fibers after 5 and 10 sec. tetanizations. (Frog sciatic nerve, 2/24/30.)

After prolonged activity excitability often followed a steadily rising course (cf. Fig. 3 and 4) reminiscent of the delayed recovery seen by Graham and Lorente de Nó (1938, Fig. 5) in perfused nerves after very short periods of rapid tetanization. But in a number of nerves the rate of recovery was increased by the development of supernormality. Figure 6 shows 3 recovery curves of this type in a nerve which had been in the moist chamber for 2 hr. (dots), 2 hr. 49 min. (circles) and 3 hr., 11 min. (crosses). In these three experiments a tetanus of 15 sec. duration decreased excitability to from 81 to 84 per cent of its resting value. A marked supernormality with an early peak

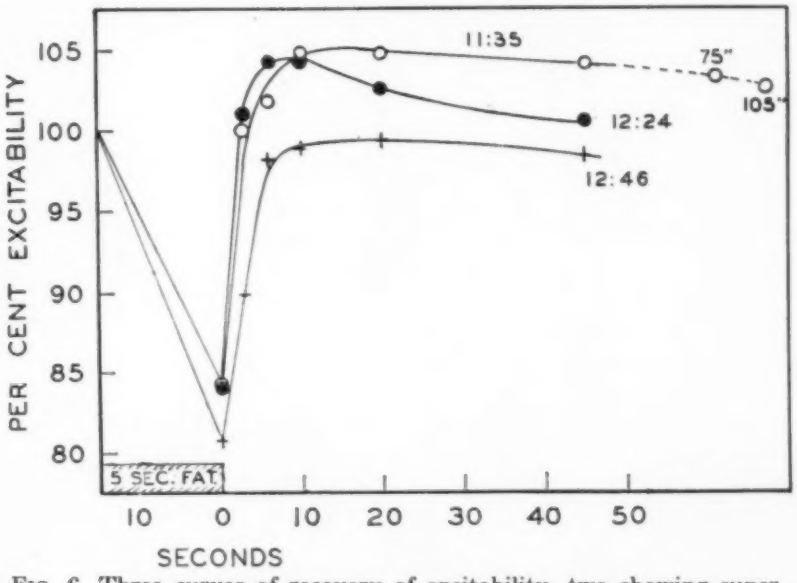

FIG. 6. Three curves of recovery of excitability, two showing supernormality. (Frog sciatic nerve, 10/4/40. Temp. 24.1°C.)

of excitability reaching not quite 105 per cent at about 8 and 15 sec. respectively after the end of the fatiguing stimulation is seen in the curves indicated by dots and by circles. In the last curve (crosses) no real supernormality is to be seen, but the rapidity of recovery considered in the light of the two other curves indicates that here also the process underlying supernormality is present.

In two experiments we had the opportunity of following the course of supernormality in recovery after tetani of varying durations. The curves of Fig. 7 illustrate one of these cases. They show also a subnormal phase following supernormality. As the duration of the fatiguing stimulation was increased, the appearance of the supernormal peak and of the subnormal depression was more delayed. This fact could only be indicated qualitatively in our experiments, since the intervals between successive tests could not be made shorter than 2.5 sec. After fatiguing stimulations of 2, 5, 15 (and 30) sec. durations the peaks of supernormality occurred at about 3, 5 and 7 sec.

after the end of fatigue; the subnormal depressions reached the minimum at about 6, 10 and 25 sec.

Gasser and Grundfest (1936) described the polyphasic return of excitability after tetanic stimulation in mammalian A fibers. From their Fig. 15 it appears that they observed the peak of supernormality to occur somewhat earlier than we found it in frog nerves as shown in Fig. 7. Their statement

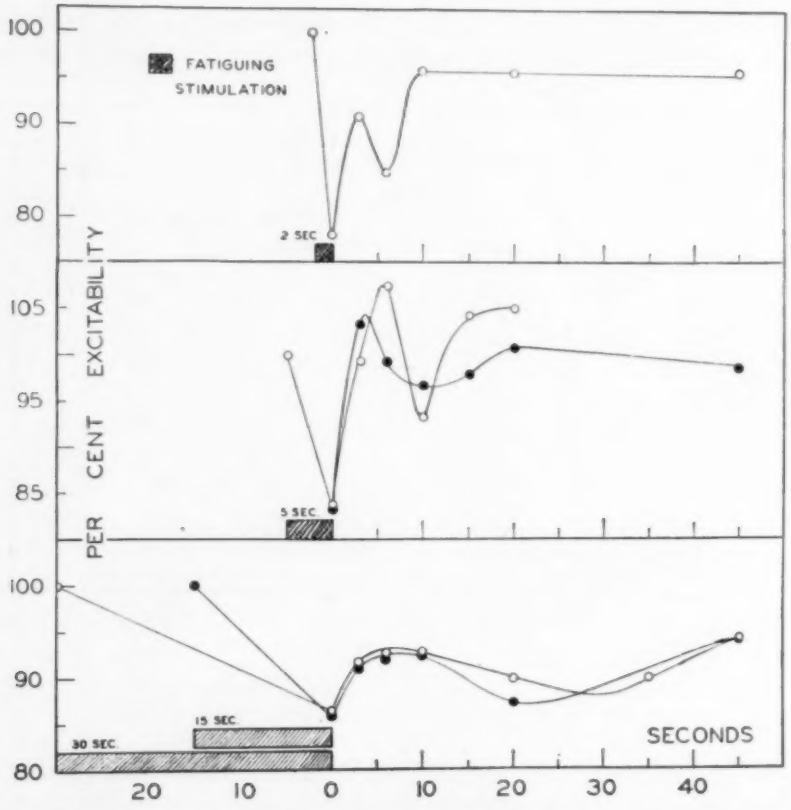

FIG. 7. Recovery of excitability in the same frog sciatic nerve after 2, 5 (dots and circles), 15 and 30 sec. tetanization (240 per sec.) showing increasing delay in the appearance of supernormality and subnormality. Nerve in moist chamber at 2:15; curves in above order taken at 2:50, 2:55, 3:00, 3:08, and 3:15 (10/10/40. Temp. 24°C.)

that the course of recovery partly depends upon the degree of previous activity is well illustrated by our results.

Recovery of excitability in different nerve fibers after activity—in spite of individual differences—on the whole shows the same trend. This is true for recovery after a single response as well as after a longer repetitive action. As a rule the following periods may be distinguished: (i) absolute refractoriness, (ii) start of recovery, (iii) supernormality, (iv) subnormality, (v) return to the normal (with occasionally a few further oscillatory changes occurring before complete recovery). The main difference between different nerves and

between nerves under different conditions lies in the spacing of these periods in time. For instance, let us follow in different nerves the time elapsing between the (last) conditioning response and the summit of the consequent supernormal period. It lasts about 7 msec. in mammalian A fibers (Gasser and Grundfest, 1936), 5 to 10 msec. in frog A fibers (Graham, 1934; Hayasi and Rittler, 1934), about 25 msec. in the sympathetic fibers for the frog

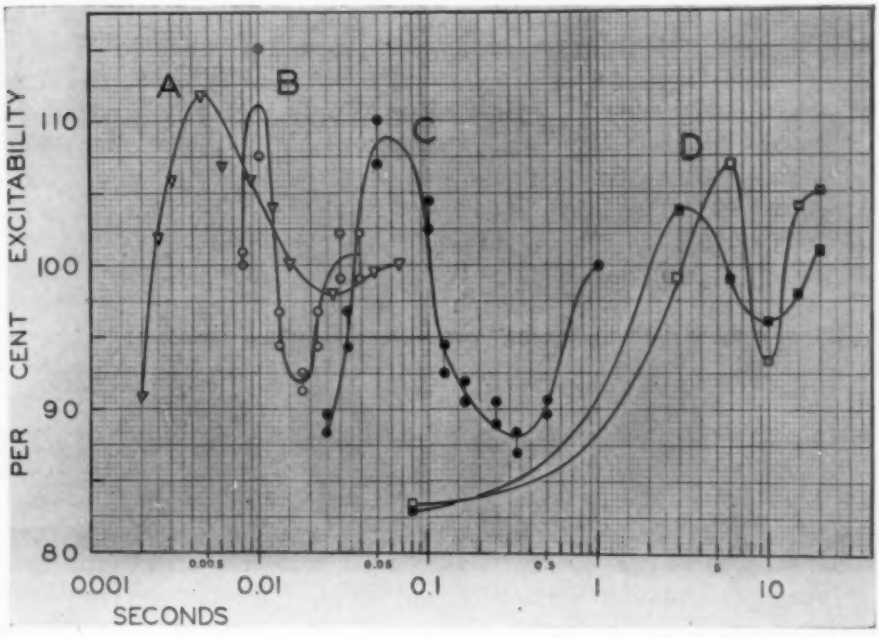

FIG. 8. Recovery of excitability with supernormal and subnormal phases in different nerves.

A. Saphenous nerve of the cat *in situ* (taken from Gasser and Grundfest, 1936, Fig. 18, top curve).

B. Sciatic nerve of the frog.

C. Preganglionic sympathetic pupillomotor fibers of the cat. B and C were drawn from chronaxie measurements by Hayasi and Rittler (1934, Fig. 2 and 4); their ordinates in this presentation therefore are arbitrary.

D. Sciatic nerve of the frog after 5 sec. stimulation (240 per sec.); both experiments on the same nerve (taken from Fig. 6 of this paper).

heart (Hayasi and E. Th. Brücke, 1934*), about 50 msec. in the preganglionic sympathetic fibers for the pupil in the cat (Hayasi and Rittler, 1934; F. Brücke, 1934), and 3 to 15 sec. in fatigued frog nerves (present experiments). Figure 8 includes in a single survey curves from these various papers. Some were taken from experiments in which chronaxie of different nerves was studied, using stimuli of different frequency. Such measurements

* It may be mentioned here that at the time these experiments were performed in my laboratory the phase of increasing duration of chronaxie was mistakenly connected with supernormality, while it is now evident that supernormality corresponds to the period with the *shortest* chronaxie.—E.B.

of chronaxie proved a very useful method of mapping the course of excitability.

Recovery of excitability after two conditioning stimuli. Whether or not late subnormality is simply a prolongation of the relative refractory period was discussed by several authors (Graham, 1935; Erlanger and Gasser, 1937; Brücke, Early and Forbes, 1941). It was pointed out that quick recovery of responsiveness during the refractory state favors an affirmative answer (Brücke, Early and Forbes, 1941, p. 90). But there still remained one objection to this assumption: Experiments with repeated stimulation (Kato, 1926) seemed to show that a nerve stimulated during its relative refractory period "recovers from the second response along exactly the same curve as if there had been no preceding response." In other words, refractoriness would not sum, whereas subnormality from successive stimulations does (Graham, 1935). As far as we see, Kato, working with nerve-muscle preparations, did not map the course of recovery of excitability in the "first" and "second" refractory state, but with a stimulus 10 times maximal measured the length of the "second least interval" at which the third stimulus would give a "summated" response. This may have revealed not the threshold of the nerve but the stage of recovery at which the subnormal nerve impulse could excite the muscle.

It therefore appeared necessary to make a comparison of recovery of excitability after a single conditioning impulse with the recovery in the so-called "second" refractory state (that is, during refractoriness following a response which falls itself in the relative refractory state of an earlier maximal impulse). The usual recovery curve of excitability was taken in isolated frog nerve, single submaximal testing shocks being applied at varying intervals after single conditioning shocks. Recovery in the "second" refractory state was observed in experiments in which *pairs* of maximal conditioning shocks separated by a fixed interval were followed by single submaximal testing shocks after the same intervals as in the first procedure. Although the "first" interval, that between the two conditioning shocks, was held constant for a given experiment, in different experiments it was varied between 1.3 and 5.5 msec. The variation of the "second" interval, between the second conditioning shock and the testing shock, furnished the points on the recovery curve. Both conditioning shocks were 5 to 10 times maximal; the strength of the submaximal shock was adjusted to give a response about $\frac{3}{4}$ of maximal in resting nerve. Excitability at different points along the curves was measured directly by increasing the strength of the testing stimulus at each interval until it evoked a response equal to the chosen magnitude of submaximal response in resting nerve. In these experiments a marked difference between recovery of excitability in the "first" and in the "second" refractory state was always found. This may be seen immediately from the different heights of response obtained at the same testing intervals in both refractory states, those in the "second" being smaller than the ones in the "first."

In Fig. 9 photographs of four pairs of responses are reproduced, the upper

row (A to D) showing recovery of height of the conditioned spike in the "first" refractory state, the lower row (A′ to D′) recovery during the "second" refractory state. The "first" interval in the pictures A′ to D′ was 1.7 msec., the "second" intervals used in the four pairs of tests were 2.7, 4.4, 5.7 and 9.3 msec. The submaximal testing shock was subthreshold for the β fibers. The response evoked by the testing shock alone (Fig. 9E) lacks the slight β elevation clearly seen in the descending part of the first conditioning spike. The second conditioning shock (A′ to D′) by summation excites a set of fibers with low conduction velocity eliciting a small potential following the second spike. In the descending part of the first spike the artifact of the second conditioning shock is seen. Using a "second" interval of only 2.7 msec.

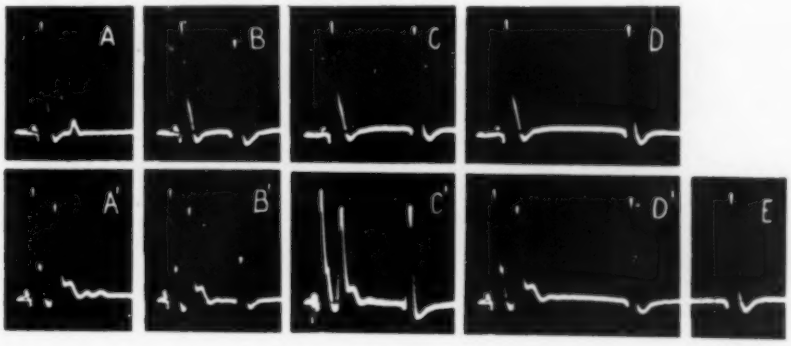

FIG. 9. Recovery of spike during "first" (A to D) and "second" (A′ to D′) refractory states. Intervals between conditioning and testing shocks in A to D: 2.7, 4.4, 5.7, and 9.3 msec. In A′ to D′ intervals between first and second conditioning shocks, 1.7 msec.; intervals between second conditioning and testing shocks as in A to D. E is the submaximal response to the testing stimulus in unconditioned nerve. (Frog sciatic nerve, 12/11/40.)

the small conditioned spike is very distinct in A; whether one of the two very small potentials in A′ is due to the testing stimulus cannot be decided. In B and B′ the difference between the responses to the test shocks is evident. In C and D measurements show that the doubly conditioned spikes are still 7 per cent and 3 per cent smaller than the singly conditioned ones. In this experiment 97 per cent excitability was reached in the "first" refractory state at about 3 msec. after the testing shock and in the "second" refractory state after about 9 msec.

As the "first" interval was increased, recovery in the "second" refractory state was found to become faster (Fig. 10A and B). Only 8 frogs were used for these experiments, a number perhaps too small to allow a definite general statement as to the length of the "first" interval at which the two recovery curves become identical. With intervals between 1.3 and 2.7 msec. recovery always was found to be slowed down. In one frog at a "first" interval of 3.3 msec., recovery already had reached its normal duration. In another experiment on a nerve which showed very slow recovery, after a "first" interval of 5.5 msec. excitability recovered only slightly more quickly than after a 1.6

msec. interval and much more slowly than during the "first" refractory phase. The time required for *full* recovery of excitability in the "second" refractory state varied widely. In Fig. 10A full height of the response was attained after about 13 msec., while in B excitability evidently achieved its resting value much later. Excitability in these experiments was not tested at intervals longer than those necessary for full recovery; therefore it is not known whether or not supernormality was present under the given condi-

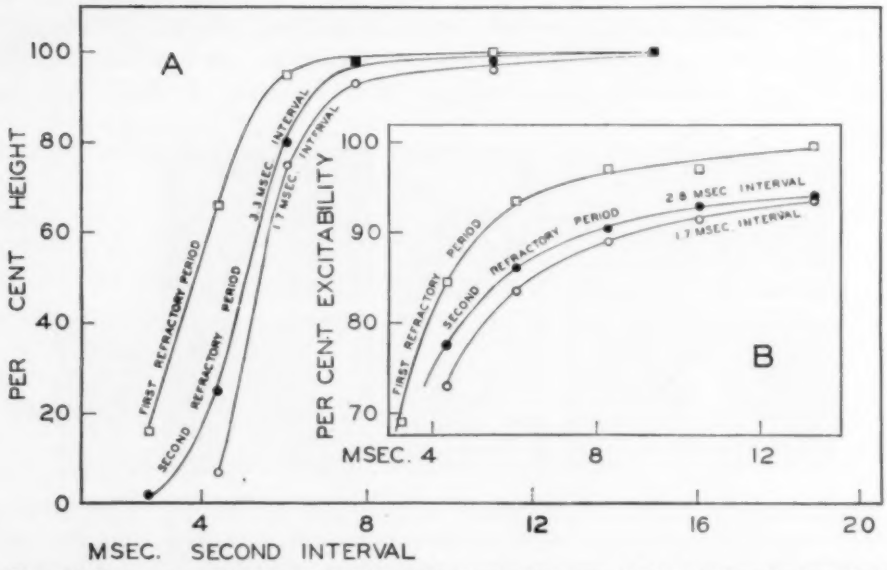

FIG. 10. Recovery of height (A) (Frog No. 2 sciatic nerve, 12/11/40) and of excitability (B) measured directly (Frog No. 3 sciatic nerve, 12/11/40) in the "first" and the "second" refractory states; testing stimuli submaximal. The intervals between the first and second conditioning shocks in the four experiments on the second refractory period are indicated on the curves.

The zero point in time for the curves of the first refractory state is at the moment of the conditioning shock; for the curves of the second refractory states it is at the moment of the second conditioning shocks. The curves have been drawn in this manner to facilitate comparison.

tions. The law according to which refractoriness increases when evoked by two, three or *n* conditioning stimuli has not yet been studied. However, it certainly depends upon the interval between the stimuli.

According to our experiments two conditioning stimuli altered recovery of excitability qualitatively in the same way as three or more stimuli did in the experiments of Graham and Lorente de Nó (1938), but in our excised frog nerves excitability was affected early in the refractory period, whereas in blood-perfused mammalian nerve this influence appeared only in the later part of recovery.

Recovery of responsiveness. The difference between the rates of recovery of responsiveness and of excitability observed in nerve by Graham and Lorente de Nó (1938) is still more accentuated in *fatigued* nerve. This may

be illustrated by the curves in Fig. 11. These curves show the height of the second response at different intervals after a maximal conditioning impulse. The recovery of responsiveness—shown in the two curves at the left—was tested with a *strong* stimulus, exciting *all* the fibers during the relative refractory state. In the unfatigued nerve the spike potential reached its full height after 2 msec. After the nerve was tetanized for 10 sec., recovery occurred somewhat more slowly, being complete after an interval of about 2.7 msec. The other two recovery curves give the height of the second response in

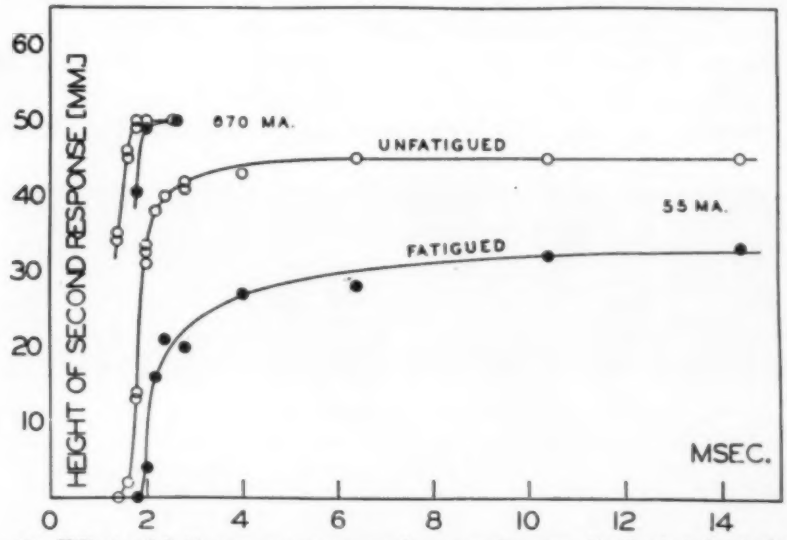

FIG. 11. Effect of fatigue on recovery of responsiveness (indicated by height of response to maximal testing shocks) and on recovery of excitability (indicated by height of submaximal spikes). Circles, recovery in unfatigued nerve; dots, after 15 sec. tetanization. (Frog sciatic nerve, 1/25/40. Temp. 24.6°C.) Strength of current in primary coil is indicated in the figure in milliamperes.

the unfatigued and the fatigued nerve tested with a just *submaximal* stimulus. Recovery of excitability in the unfatigued nerve is complete after 5 msec.; in the fatigued nerve the separation of recovery of excitability into two parts (Graham and Lorente de Nó, 1938) is marked and complete recovery occurs late.

When using unconditioned *supermaximal* testing shocks in studying recovery after prolonged activity in many nerves we met with higher responses immediately (80 msec.) after the end of the fatiguing stimulation than at 2 or 3 sec. later. This abnormally strong reaction to the first testing shock after the fatiguing stimulation was associated with a shifting of the baseline in the positive direction. In a few experiments testing shocks of 2 to 8 times maximal were used; the increase of height immediately after tetanization became more marked as the stimuli were strengthened. With weak testing stimuli, however, a small response or even none at all was seen, indicating that the abnormal height of response to the first supermaximal stimulus was not due

to increased excitability but to increased responsiveness. In Fig. 12 the second of the six spikes shows both the shifting of the baseline and the abnormal height of the spike; in the same figure changes of conduction velocity due to fatigue may be seen directly from the shape of the β wave. The relation of these findings to those reported by Forbes and Rice (1929) has not been thoroughly investigated. It is possible that their decreased size of response following the fatiguing stimulation was due to decreased excitability causing the stimuli to become slightly submaximal.

In some experiments in which testing stimuli of *medium* strength were used, the unconditioned response immediately after a fatiguing stimulation was smaller than before the tetanization, but it was not really reduced to the minimum, as the next testing stimulus, after 2 or 3 sec., provoked a still smaller response (cf. Fig. 13). It seems as if in these cases the dropping out

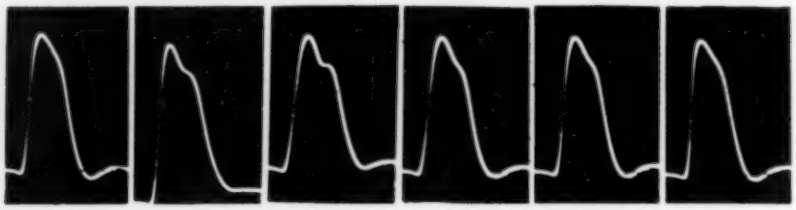

FIG. 12. Responses to supermaximal testing stimuli. From left to right: spike in resting nerve, spikes recorded at 80 msec., 2.5, 7.5, 10, and 12.5 sec. after 15 sec. fatiguing stimulation (240 per sec.). Testing stimulus 5 times maximal. (Frog sciatic nerve, 10/30/40.)

of some fibers because of decreased excitability was partly compensated for by an increase in responsiveness in the still responding fibers.

Since anodal polarization has long been known to increase the amplitude of the spike potential (cf. Bishop and Erlanger, 1926) we thought that perhaps a direct-current component in our fatiguing stimuli might be the cause of the phenomenon. Therefore in later experiments the fatiguing shocks delivered by the photocell were sent through a transformer before reaching the nerve. But with this arrangement, also, abnormally high responses were seen immediately after the end of the tetanization. There is also a possibility that the increasing spike magnitude corresponds with a period of positive after-potential after the fatiguing repetitive stimulation; no experiments were carried out to test this possibility. No abnormal height of response was observed during the refractory period set up in a fatigued nerve by a conditioning shock. The effect of the second (testing) shock in these experiments 80 msec. after the end of the fatiguing stimulation was always smaller than it was some seconds later.

Marked prolongation of refractoriness in fatigued nerve was first described by Field and Brücke (1926), who at that time thought that the *absolute* refractory state also was prolonged by a fatiguing stimulation. Woronzow (1935) later found an increase in duration of the absolute refractory

state (from 1½ to 3 times its resting value) only after several hours' tetanization. He justifiably criticized Field and Brücke for having used stimuli that were below threshold for the most excitable fibers in the early relative refractory state. But Woronzow's statement that the *relative* refractory state also was not much prolonged after fatigue is probably due to the fact that he used *strong* testing stimuli, by which he studied the relatively quick recovery

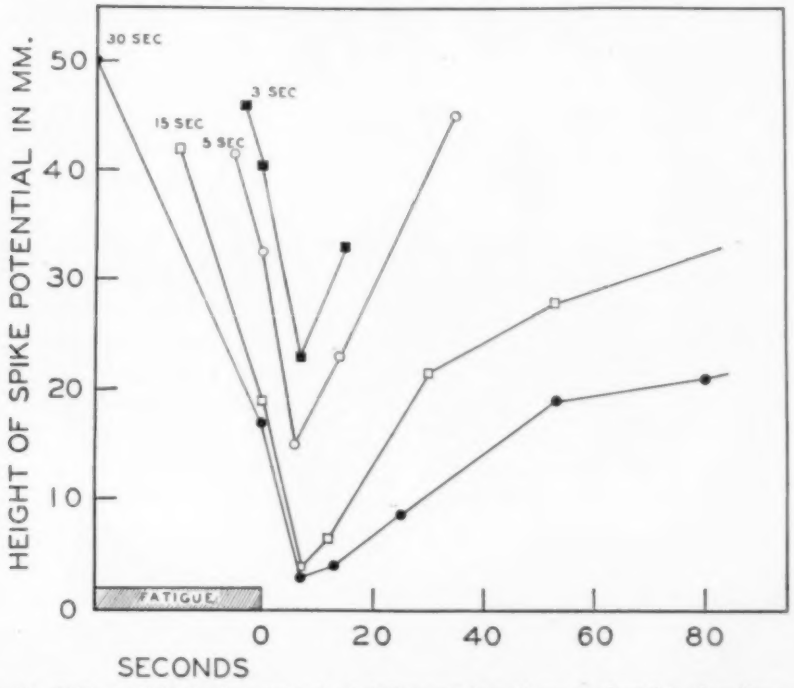

FIG. 13. Effect of fatiguing tetani of different durations on height of spike potential. Note that the first response after the end of fatiguing stimulation is higher than the next one following after about 5 sec. Rate of recovery parallels duration of tetanus. (Frog sciatic nerve, 1/15/40.)

of responsiveness rather than the recovery of excitability. It can no longer be doubted that recovery of excitability in nerve is greatly delayed by fatigue.

DISCUSSION

In the present paper it seemed advisable not to discuss experimental results in a separate section, but to include the discussion with the observed facts. Only a few points therefore will be mentioned separately here.

Nerve and heart. For the study of many physiological problems the heart may be used as a model for excitable tissue, but as far as its refractory state is concerned, it seems to behave in a slightly different way from nerve. The refractory state following an extrasystole is not prolonged, but shorter than the one after a normal systole (Trendelenburg, 1903; Umrath, 1925). Whether contractility of the heart muscle recovers during the relative re-

fractory state with the same speed as excitability, has not, as far as we know, been definitely determined. The data presented by Tigerstedt (1921) suggested that in the frog's ventricle excitability recovers more quickly than does the height of systolic contraction.

Refractory and subnormal periods. The period during which excitability returns to normal after a single response is called the "relative refractory period." Graham and Lorente de Nó (1938) from their experimental findings —confirmed in the present paper—suggested a division of the relative refractory state into two parts "during the first of which (1.5 to 2 msec.) height and velocity recover completely, and excitability recovers to an extent (roughly 80 per cent) that does not change significantly after rhythmic activity. The completion of the recovery of excitability during the second part of the cycle is greatly affected by rhythmic activity and other conditions." This second part of recovery—if followed by a supernormal phase—may end at the point where supernormality starts. On the other hand, the increase in refractoriness after a second conditioning stimulus and the fact that responsiveness increases no further during the second part of the refractory period are in favor of the assumption that *subnormality*, when observed after the supernormal period, is not to be considered a separate phenomenon; it it just a late continuation of recovery of excitability (cf. Brücke, Early and Forbes, 1941). Thus the refractory state may last for many seconds, even for minutes, yet the process of which it is the expression may be the same after a single nervous impulse as after prolonged activity (cf. Fig. 8). If the idea is correct that recovery of *excitability* during the refractory and the subnormal period is one and the same process, the difference in time between this recovery process and the quick restitution of responsiveness becomes still more marked. It might be useful to separate them by nomenclature. The term "relative refractory period" might be reserved for the first short period of recovery of responsiveness, whereas the expression "subnormal period" (Graham, 1935) already used for a relatively slow recovery of excitability, might be reserved for this process as a whole in whatever form it appears.

A difference between relative refractoriness after a single impulse and the slow recovery of excitability after prolonged activity may be seen in the fact that no positive after-potential has been found to accompany the relative refractory phase as it does subnormality after prolonged activity. This difference, however, is of hardly any importance because the positive potential in A fibers is so small that it could not be detected if it were present at the beginning of the relative refractory period, that is, during the steep descending part of the spike, and it could easily be masked by the negative potential at the end of the spike.

Elementary manifestation of fatigue. The depression in excitability due to prolonged stimulation is the main and often the only symptom of the condition that is generally called "fatigue" in nerve. Since this condition affects and prolongs the subnormal period which is assumed to be the same as the old "refractory" period, it is merely a matter of nomenclature whether we speak of a state of fatigue or of a prolongation of the refractory period. The delay of recovery during the "second" refractory state may be the first detectable sign of fatigue. But in going one step further we might also assume

that not only is *prolonged* refractoriness a symptom of fatigue in nerve but also that the short refractory state itself following a single impulse in resting nerve is the elementary manifestation of fatigue.

Summary

Recovery of excitability of frog sciatic nerve was studied after one and two conditioning shocks, after prolonged tetanization and in experiments combining prolonged activity and refractoriness. Generally excitability was measured by the method of Graham and Lorente de Nó (1938), the theoretical foundation of which is discussed.

To compare the effect of similar fatiguing tetanizations on submaximal single responses and on conditioned responses the testing stimuli were adjusted before fatigue so that conditioned and unconditioned responses would involve the same number of fibers. It was found that excitability was reduced much more in the unconditioned (resting) than in the conditioned (refractory) nerve. In both cases the rate of recovery of excitability depends directly on the duration of the fatiguing stimulation.

Fatigability does not vary in different α fibers of a given nerve.

The appearance of supernormality and of subnormality was delayed by fatiguing stimulation, the degree of delay depending upon the degree of previous activity. Recovery curves of different nerves taken from various papers were plotted together on a semi-logarithmic scale. Thus in a single survey the different spacings in time of supernormality and subnormality are revealed. In a variety of preparations although the total duration of the recovery curve varies greatly, the sequences are similar (Fig. 8).

Recovery of excitability after a single conditioning impulse was compared with recovery during the so-called "second" refractory state (after two conditioning impulses). Recovery was always found to be slowed down in the latter case; as the interval between the two conditioning impulses was increased, recovery in the "second" refractory period was found to become faster. This delay of recovery during the "second" refractory period may be the first detectable sign of fatigue.

The fact that the separate refractory states after two impulses sum, just as subnormality does, and the fact that responsiveness increases no further during the second part of the refractory period (Graham and Lorente de Nó, 1938) are considered to favor the assumption that subnormality is a late continuation of the relative refractory state.

The difference between the rates of recovery of *responsiveness* and of excitability is accentuated in fatigued nerve.

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ACETYLCHOLINE ESTERASE CONCENTRATION DURING THE DEVELOPMENT OF THE HUMAN FETUS

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INTRODUCTION

PREVIOUS studies (Youngstrom, 1938) have shown a definite correlation between the choline esterase concentration and the functional development of the neuromuscular system in amphibian embryos. The present work is an extension of these studies to the human fetus which, because of its large size and slow development, is admirably suited to the purpose.

The specimens used were made available through the coöperation of the Department of Obstetrics and Gynecology, and were selected from cases of therapeutic abortion (hysterotomies and hysterectomies) with due regard to their behavior as established by personal observations and by comparison with the descriptions of human fetal behavior published by Hooker (1936, 1939). The pharmacological assays performed upon their tissues have provided evidence of a correlation between the choline esterase content and the functional development of the nervous and muscular systems.

Nachmansohn (1940a) has studied the choline esterase content of several species of animals, giving data on rather scattered periods of their developmental life. In so far as his works have a bearing on the present study, they will be discussed later.

MATERIAL AND METHODS

As soon as a specimen was received from the operating room it was placed in warm saline for the exploration of its behavior and subsequent dissection. The parts selected for esterase determination were placed in individual Wassermann tubes in an icebox at 0°C.* until such time as the esterase determination was to be done.

The tissue was then ground in a Ten Broeck grinder with a definite amount of buffer of pH 7.61 and diluted with distilled water to a standard suspension. The amount of esterase was determined by the pharmacologidal method with the guinea pig ileum (Bernheim and Bernheim, 1936). Eight specimens were used which ranged in size as follows: 21, 33, 48, 51, 65, 100, 135, and 240 mm. live crown-rump length, respectively. Their menstrual age was calculated from their crown-rump length by applying the formula of Edith Boyd.†

RESULTS AND DISCUSSION

The results are presented in Fig. 1, each plotted point representing the acetylcholine esterase concentration of a particular part of one fetus. The

* As previously reported by Mann, Tennenbaum and Quastel (1939), the enzyme was found to be stable at this temperature.

† $A = \frac{83.259015}{2.820106 - \log L}$, where A is menstrual age in days and L is crown-rump length in millimeters (quoted from Hooker, 1936).

unit of enzyme activity is the number of mg. of acetylcholine iodide hydrolized by 100 mg. of tissue (dry weight) per hour at pH 7.61 and 38°C.

The youngest human fetus in this study, being approximately 56 days menstrual age, is almost ready to move (Hooker, 1936). The rapid expansion of behavior immediately following this period is paralleled by an equally rapid increase of the choline esterase concentration in the spinal cord, medulla and midbrain, in other words the region of the motor plate. This increase continues at least to 121 days by which time the fetus has almost all the specific reflexes of the adult except respiration (Hooker, 1936). The sequence by which the enzyme was found to increase in the different parts of the central nervous system (midbrain and medulla, spinal cord, diencephalon, basal ganglia and cerebral hemispheres) correlates very well with the order of morphological differentiation in the central nervous system (Coghill, 1929; Angulo, 1939). It is also in general agreement with the four phases of fetal development as outlined by Minkowski (1938) from his study of the response to plantar stimulation; however the present data suggest that functional development begins somewhat earlier in the several regions of the central nervous system than is indicated by him.*

In the case of the basal ganglia and of the cerebrum, there is but slight increase in the choline esterase concentration until after 121 days menstrual age, when a precipitous increase occurs. This suggests that differentiation of this region does not really get under way until after four months menstrual age, which agrees very well with the morphological differentiation in the human cerebral cortex as it has been described by von Economo and Koskinas (1925).

Because of its late development and small size, no determinations have been attempted with the cerebellum until 102 days menstrual age. By 189 days its choline esterase concentration has more than doubled and has almost reached the value for the adult cerebellar cortex.† The most rapid growth of the cerebellum comes after the seventh fetal month (Dunn, 1921).

The liver was the most concentrated source of choline esterase in the 56-day fetus. The highest values for this tissue were obtained when specimens between 56 and 75 days menstrual age were used. This is during the period when hemopoesis is most active in the liver (Knoll, 1932), a fact which takes increased significance in view of the report that choline esterase is more concentrated in the cells than in the serum of the blood, and that the choline es-

* After the menstrual age of his fetuses has been calculated by applying Boyd's formula to their crown-rump length, his four phases would then be as follows: (i) Neuromuscular transitional phase when the influence of the spinal cord and medulla is beginning to be exerted upon the muscles (68–74 days); (ii) The spino-bulbar phase (72–129 days); (iii) Tegmento-bulbo-spinal phase (135–243 days); and (iv) Pallido-rubro-cerebello-segmento-spinal phase (243 days till birth). The menstrual ages actually assigned to these periods by Minkowski are (i) about two months; (ii) 3 to 4 months; (iii) 4 to 6 months; (iv) 6 months to birth.

† The following results on adults were obtained with material secured from neurosurgical cases through the coöperation of Dr. Barnes Woodhall: cerebral cortex 2.2, 0.9, 2.0, 2.1; internal capsule 1.18, 1.5; cerebellar cortex 28.

terase of the cells is qualitatively different from that of the serum (Alles and Hawes, 1940). It is apparently the red cells with which the enzyme is associated since washed pus cells (Ginsberg, Kohn and Necheles, 1937) and a practically pure mass of histiocytes from an area of a brain tumor* contained almost no esterase. The formation of bile salts in the fetal liver may be another factor in the eventual depression of this curve (Glick, Lewin and Antopol, 1940).

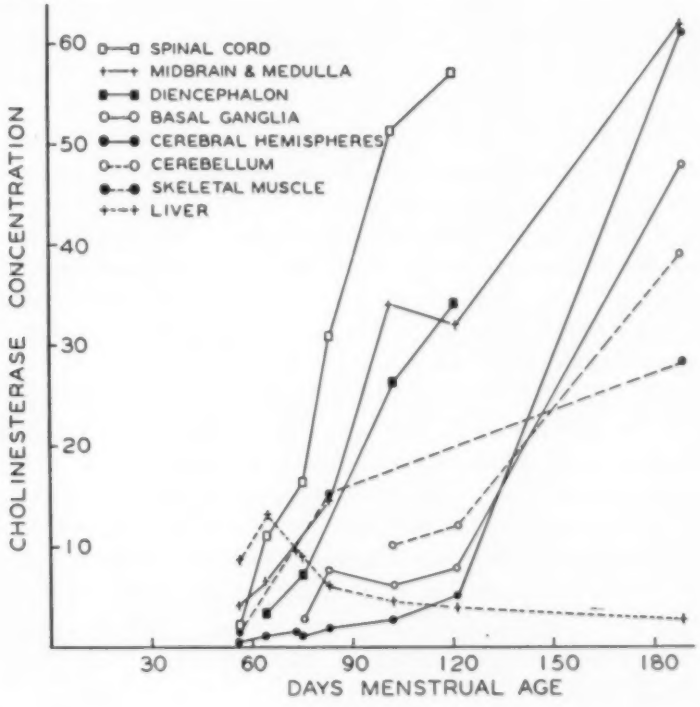

FIG. 1.

The most rapid concentration of choline esterase in the muscles of the back and shoulder comes when motility is developing in these parts (Hooker, 1936). This is also the period when the enzyme is being concentrated most rapidly in the spinal cord. Thus there is seen a close relationship between the period of rapid increase of choline esterase and the functional development of the neuromotor mechanism.

Since the endplates are not yet present (Dickson, 1940) when the skeletal muscles of sheep fetuses have the richest concentration of acetylcholine esterase, Nachmansohn (1940b) concluded that "the concentration is more closely related to the function than to the histological formation" (p. 401). He has not attempted precise correlation of the enzyme concentration with the developing behavior of the animals studied by him. Though spontaneous

* Unpublished observation.

movements begin as early as 34 days gestation in sheep fetuses (Barcroft and Barron, 1939) no data are given on the choline esterase content earlier than 60 days. In so far as correlations are possible, his results are in general agreement with the studies presented herewith on the human fetus.

Windle and Fitzgerald (1937) state that the spinal reflex arcs of the human are completed during the 8th week of intrauterine life; however, it appears difficult to demonstrate motility in the human fetus during and somewhat after, this period. Furthermore, there is no general agreement as to the correct interpretation of the movements observed. Some insight into this difficult problem is gained from a consideration of the neurohumoral mechanisms at this stage of development.

Kuo (1939) has shown that the most rapid increase in the concentration of acetylcholine in the chick embryo comes between the second and fifth day of incubation after which the concentration may be said to remain at the same general level if allowance is made for considerable fluctuation. Thus the acetylcholine concentration in the chick embryo has practically reached its maximum before there is any behavior. It would appear therefore, that motility may be hampered in its development by a deficiency of the enzyme choline esterase rather than of the substrate, acetylcholine.

Assuming that acetylcholine is liberated by certain neurons as they begin functioning in the human fetus, the removal of the products of the action of choline esterase (acetic acid and choline) by the circulation may be at first deficient in some respect as has already been indicated in amphibia (Youngstrom, 1938). Furthermore, the proportion of electrolytes in the early stages may affect the choline esterase activity (Mendel, Mundell and Strelitz, 1939). Thus the accumulation of high concentrations of choline esterase in the fetus might be thought of as a compensatory mechanism. A clearer understanding of this mechanism should lead to a broader comprehension of developing motility and behavior.

Summary

1. The acetylcholine esterase concentration in the several gross divisions of the central nervous system, in skeletal muscle and in liver has been determined on the human for the fetal period.

2. Correlation of these studies with the developing motility and behavior reveals a significant relationship. The period of most rapid concentration of choline esterase in the several gross divisions of the central nervous system seems to parallel their functional development.

3. The liver has its highest concentration of choline esterase in the early fetal stage.

4. A satisfactory explanation has not yet been found for the occurrence of a greater concentration of choline esterase in fetal than in adult tissues.

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LOCALIZATION OF CEREBRAL CENTER ACTIVATING HEAT-LOSS MECHANISMS IN MONKEYS*

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THERE is considerable evidence to indicate that an elevated intracranial temperature constitutes an effective stimulus for the activation of heat-loss mechanisms. The supposition is that the increase in the degree of cerebral heat is due to a rising temperature of the blood, a premise which has received substantial experimental indorsement. Warming of the carotid blood has been shown to cause sweating, peripheral vasodilatation and polypnea in various animals (Kahn, 1904; Moorhouse, 1911; Hammouda, 1933). This testimony has been upheld by the results obtained on direct heating of the brain, whether by circulating hot water through a closed tube inserted into the region under investigation or by open irrigation with warm fluids. By such methods, several workers have produced a fall in body temperature, sometimes accompanied by vasodilatation (Barbour, 1912; Hashimoto, 1915; Prince and Hahn, 1918).

In these early researches the corpus striatum was thought implicated and adjudged to be the "heat center." However, Sachs and Green (1917) could not confirm this hypothesis, while Moore (1918), though able to induce an antipyretic effect by warming the forebrain, determined that the corpus striatum was not specifically responsible. In the light of subsequent studies, it is probable that the positive results elicited from the striate body were due to the spread, to other areas, of the prolonged and intense heat applied in these experiments.

Attention was directed to the possible rôle of the hypothalamus and adjacent structures by Hasama (1929), who provoked profuse sweating and a fall in body temperature by warming the base of the brain in this vicinity in the cat. Supportive though inconclusive evidence was provided by Hammouda's (1933) demonstration that injection of warm saline into the third ventricle caused panting in the dog. In an attempt to delineate the heat-sensitive region with greater precision, Magoun, Harrison, Brobeck and Ranson (1938) resorted to the more refined technique of heating the brain locally by a diathermy current passed between electrodes placed at desired loci with the Horsley-Clarke instrument. They outlined, in the cat, a discrete field, heat-stimulation of which caused respiratory rate increase, panting and sweating. This center was situated between the anterior commissure and the optic chiasma, and there was a zone of lesser response extending caudally through the diencephalon to the midbrain. In 1940, Hemingway,

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Rasmussen, Wikoff and Rasmussen essentially repeated the experiment of Hasama (1929), heating the base of the brain in the unanesthetized dog with a high-frequency current conducted by a plate electrode implanted over the optic chiasma at a previous operation. They were thereby able to inhibit shivering and induce vasodilatation, but not to obtain panting.

The present study on the monkey was undertaken in order to examine the problem in an animal of a higher order so as to throw more light on the question of the central control of body temperature in man.

METHODS

Eighteen monkeys (Macaca mulatta) were employed in this investigation. Eleven of these were used only incidentally; they were normal animals of the colony which were subjected, for varying lengths of time, to environmental temperatures of 104° to 120°F., in order that the typical heat-loss behavior of the monkey might be determined.

Seven macaques were utilized for experimental heating of the brain. Anesthesia was produced by urethane, 0.8 gm. per kg. of body weight, injected intraperitoneally and occasionally supplemented with ether during the surgical preparation of the animal. No polypneic breathing, as is common in the cat under the influence of this narcotic, was observed in the monkey. Operative exposure was provided by a trephine opening made in the midline of the calvarium in the region of the frontoparietal suture and enlarged with rongeurs. The dura mater was widely incised and reflected. So that the midline might be explored, the superior sagittal sinus was in some instances compressed with a small metal clip to reduce its width and clear the field for introduction of the electrodes.

Two straight needle electrodes, 0.5 to 1.0 mm. in diameter, were mounted in the multiple holder of the Horsley-Clarke instrument, and the distance separating them in the transverse axis adjusted as desired between 2 and 8 mm. The electrodes were constructed either of drawn nichrome wire or of copper wire, insulated, respectively, with enamel or with thin-walled glass capillaries sealed to the copper, to within 2 mm. of their pointed tips. Orientation of the electrodes within the brain was achieved by use of the stereotaxic apparatus, after the technique of Ranson (1934). The electrodes were always inserted so as to be equidistant from the midline. The vertical and rostrocaudal steps in exploration were routinely spaced at 2 mm.

A tuned-plate, tuned-grid oscillator similar to that used by Magoun, Harrison, Brobeck and Ranson (1938) was employed. This delivers a current similar to that of the familiar diathermy machine, but of low power. In each experiment, an alternating voltage of high frequency (1 Mc.) and low intensity (14.2 V.) was applied to the electrodes for a 5-minute period. At conductance of the current between the tips of the electrodes and through the intervening tissue, the output voltage fell to an intensity in the neighborhood of 11.0 V. To determine how heating occurred during the passage of the current the temperature of the electrodes and of the brain substance in contact with the electrodes was measured in preliminary experiments on cats by using a thermocouple as one of the electrodes. It was found that equilibrium was established one minute after onset of current passage; a temperature gradient ceased to exist one half minute after the current was discontinued. Application of 14.2 V. to electrodes separated by 6 mm. raised the temperature of the tissue in contact with an electrode by 10.5°C. It was manifest that the temperature gradient with distance from the electrode surface was steep. Therefore, it can be assumed that a localized, approximately spherical heat field was centered about the tip of each electrode.

During each individual experiment, the amount of skin moisture, the respiratory rate and the rectal temperature were followed. Some of the work was carried out in a humidified room, in order that sweating might be more easily observed directly; the remainder under ordinary conditions of humidity. The room temperature was constant throughout, in the vicinity of 80°F. In certain animals sweating was detected by the iodine-starch indicator technique as described by List and Peet (1938). Between periods of heating the brain, the animal was allowed to rest for 5 min., or longer until sweating had ceased. The respiratory rate was counted at frequent intervals (1 min. or less). Rectal temperature was noted by thermometer or recorded continually by a Leeds and Northrup Micromax. Some attempts

were made to gauge alterations in the vasomotor state by measuring skin temperatures with locally applied thermocouples or with a Tycos Dermatherm.

The brains were serially sectioned in the plane of the punctures and stained by the Weil method. All points heated were located and plotted on a succession of projecion tracings prepared from sections at one millimeter intervals on the formalin fixed brain. From this series there were selected the levels at 2 mm. intervals reproduced in Fig. 1, upon which were also plotted the reactive points from intervening levels. In most of the specimens there was little destruction (usually much less than 0.5 mm. in radius) around the electrode tracts.

Results

Responses. The 11 normal animals exhibited stereotyped heat-loss behavior when exposed to high environmental temperatures and served to establish a norm with which the responses to local cerebral heating could be compared. As in the cat, heat-loss mechanisms were brought into play in the monkey only after a considerable rise in body temperature. In all animals the requisite rectal temperature level was slightly more than 103.0°F., at which point sweating began rather abruptly. Perspiration was marked on the palms (much more marked than that occasioned by excitement) and noticeable on the face and forehead. With the onset of sweating, flushing of the skin occurred, and the respiratory rate rose rapidly. Unlike the dog and cat, there was no panting.

By local heating of the brain at appropriate sites, heat-loss responses, identical to those exhibited by the normal animals, were produced. Palmar and facial sweating were both observed, the former being conspicuous, while the latter was distinct only when its appearance was accentuated by the iodine-starch indicator. When it ensued during heat stimulation of the brain, sweating came on slowly after about 3 min. of heating. During the control intervals of 5 min. or more between the periods of heating, no sweating was observed, though external conditions had not changed. Since perspiration was the most clear-cut response, it was used as the criterion of heat-loss activity. On the projection tracings (Fig. 1) stimulated points have been plotted as negative (dots), slight (open circles) or marked (filled circles) with regard to sweating. The differentiation between slight and marked sweating was of course arbitrary, though in most cases it was easily made.

Accompanying sweating on the heating of reactive regions was a consistent increase in the rate of respiration, an increase which began shortly before the onset of sweating. It is possible that there was minimal, nondetectable perspiration at some points, which may account for the fact that at a few sites, classed as negative, there was a moderate tachypnea. However, comparison of the respiratory rates during excitation of non-responsive points leaves no doubt as to the reality of the augmentation. For 50 negative points, the average respiratory rate before heating the brain was 54 per min., the average high attained during stimulation 59.7 per min., and the mean increase in rate therefore 5.7 per min. For 43 points, the activation of which induced sweating, whether slight or marked, the average beginning rate was 52.7, the high 76.1, and the mean advance, therefore, 23.4 respira-

tions per min. This difference in respiratory rate increment is statistically significant,* and the conclusion that local heating of certain regions of the brain produces polypnea as well as sweating is inescapable.

Though peripheral flushing was at times noted, attempts to measure changes in skin temperature proved largely unsuccessful. Some alterations were observed, but none of truly significant magnitude. It should be noted, however, that neither an ordinary thermocouple nor the Tycos Dermatherm is satisfactory for measuring skin temperature in animals under ordinary laboratory conditions. A radiometer was not available. No noteworthy modifications in rectal temperature attributable to brain heating were remarked. The usual range in rectal temperature during an experiment was from 98.6°F. to 102.0°F. At no time, when positive heat-loss responses were being obtained, were rectal temperatures above 103.0°F.

Localization. Because of the previous delimitation of the heat-sensitive region in the cat, it was not felt necessary to perform an anatomically complete survey of the brain. However, the monkey cerebrum was thoroughly and systematically explored from a level just rostral to that of the genu of the corpus callosum caudally through the tuberal level of the hypothalamus, from the midline to a plane 5 mm. lateral to it, and from the corpus callosum ventrally to the base of the brain. From only one locus within this area were heat-loss reactions induced. A region yielding maximum sweating responses (plotted as solid circles; Fig. 1B) lies in the ventrocaudal part of the telencephalon between the optic chiasma and the anterior commissure. This field has an anteroposterior extent of 1.5 to 2.0 mm., from a level just oral to the crossing of the anterior commissure to the caudal limit of the optic chiasma. The mediolateral spread cannot be fixed exactly, but presumably the sensitive area lies within planes erected parallel to the midline 4 mm. to either side of it, since, when the electrodes were separated by 8 mm. and equidistant from the midline, no sweating was obtained.

Surrounding the area just described, there is a girdle of less marked sensitivity. Restrally, the first slight sweating responses are found at the very base of the brain, at the level of the diagonal band of Broca (Fig. 1A). Laterally and dorsally to the maximally reactive region slight responses are met with (Fig. 1B), while behind it there are a few scattered minimally responsive points. Also some spots yielding slight sweating are seen ventral

* The average rise in cases of positive sweat response was 23.4 strokes per minute, for negative instances, 5.7. The standard deviation of the mean was computed for each by the formula $\sigma_m = \pm\sqrt{\frac{\Sigma[m-x_1]^2}{n(n-1)}}$, and found to be 2.09 and 0.968 respectively. The critical ratio of these two values was determined by the formula $R = \frac{M_2 - M_1}{\sqrt{\sigma m_1^2 + \sigma m_2^2}}$, and ascertained to be 7.65. Since significance is commonly assumed if R is greater than 2, the difference between the accessions of rate is regarded as highly convincing. We are grateful to Dr. F. T. Jung of the Department of Physiology, Northwestern University Medical School, for his suggestions concerning the proper statistical management of these data.

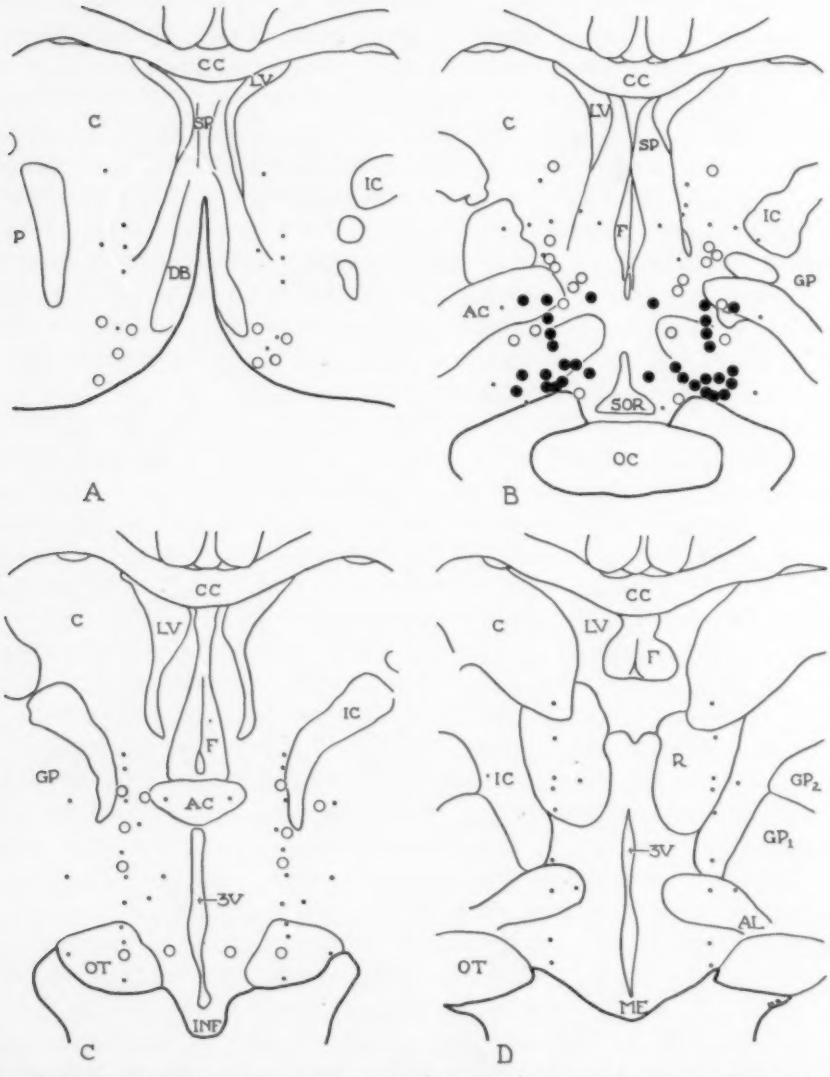

FIG. 1. Four sections at 2 mm. intervals through the preoptic area and anterior hypothalamus of the monkey. Each point on one side of the brain has its counterpart on the other, since all exploration was performed by pairs of electrodes. Symbols: dots, negative; open circles, slight sweating; filled circles, marked sweating. A level one millimeter rostral to that of A showed no positive responses. Abbreviations: AC, anterior commissure; AL, ansa lenticularis; CC, corpus callosum; C, caudate nucleus; DB, diagonal band of Broca; F, fornix; GP, globus pallidus; IC, internal capsule; INF, infundibulum; LV, lateral ventricle; ME, median eminence; OC, optic chiasma; OT, optic tract; P, putamen; R, nucleus reticularis; SOR, supraoptic recess; SP, septum pellucidum.

to the region of unequivocal sensitivity (Fig. 1B and C). The zone from which only slight sweating was elicited is in turn surrounded by areas nonreactive to heat stimulation. At levels 1 mm. rostral to that of Fig. 1A (not illustrated) and 2 mm. caudal to that of Fig. 1C (Fig. 1D), no heat-loss

activity was obtained by local heating. Points lateral and dorsal to the responsive areas are negative (Fig. 1B) as are some points ventral to it (Fig. 1B and C).

Discussion

The strict dependence of the activation of sweating and polypnea on the heating of only a certain portion of the brain and the complete absence of heat-loss behavior during control periods when no current was passing constitute a convincing demonstration of the validity of the observations reported. The inability to measure skin temperature rises is assumed to have been due to inadequate instruments and technique. The periods during which the heat-loss mechanism was activated were too short to cause a fall in body temperature.

Reasons for crediting the results to heat stimulation and not to the severance of any descending pathways from higher centers have been presented elsewhere (Magoun, Harrison, Brobeck and Ranson, 1938). The facts that in most cases there was no extensive destruction of tissue, and that the responses disappeared with the cessation of intracranial heating and were repeatable hold true for the monkey as for the cat and establish emphatic evidence against the surmise that injury might be responsible.

The outcome of these experiments does not prove that the reactive region contains the final efferent supranuclear neurons for the activation of heat-loss mechanisms; it is quite possible that it contains afferent-like elements capable of exciting efferent groups of cells situated more caudally. The interpretation placed on the results is that by artificial heating of the brain the same behavior is made operative as is normally brought into play by a rising cerebral temperature contingent upon an increased degree of warmth in the circulating blood.

The area found to be maximally susceptible to local heating in the monkey coincides closely with the anatomic preoptic region. The band of surrounding lesser responses may represent sites of less compact concentration of the sensitive elements or may illustrate spread of the heat to more specifically reactive points.

The field yielding marked sweating is comparable to that found to be heat-responsive in the cat (Magoun, Harrison, Brobeck and Ranson, 1938). The principal difference between the topographies of response in the two animals is that in the cat a zone of lesser reactivity was encountered through the dorsal hypothalamus; this feature was not present in the macaque. Hemingway, Rasmussen, Wikoff and Rasmussen (1940) have brought forth confirmatory evidence in the dog. They obtained partial activation of heat-loss mechanisms by warming the anterior hypothalamus. Their inability to produce panting was probably due to the fact that the optic chiasma and vascularized scar tissue intervened between the source of heat and the preoptic region. This distance factor must have prevented completely effective heating of the sensitive area. The authors note that a thermocouple placed 2 mm. from the electrode showed body temperature at all times.

Reinforcing the localization afforded by brain heating experiments are the studies of many workers on the results of destructions and transections of the brain-stem; these have been reviewed elsewhere (Ranson, 1940). More concrete affirmation has been given by the consequences of localized lesions in the heat-responsive center in the cat (Teague and Ranson, 1936; Clark, Magoun and Ranson, 1939) showing that its destruction results in impaired ability to regulate against environmental warmth in the chronic state. The results of destroying the heat-sensitive region in the monkey are now being studied (Beaton, McKinley, Magoun and Ranson) and the preliminary experiments confirm the findings of exploration with the high frequency current. When these results are presented the problem of neurogenic hyperthermia will be discussed.

Summary and Conclusions

Local heating of the brain of the monkey by a low-voltage, high-frequency current passed between electrodes oriented in the brain with the Horsley-Clarke technique has demonstrated a reactive field which responds by bringing into play the heat-loss mechanisms of sweating and polypnea. The probably associated vasodilatation was not demonstrated because of technical difficulties.

The area in question is located in the preoptic region of the telencephalon between the anterior commissure and the optic chiasma, with a rostrocaudal extent of about 2 mm. and a lateral reach of about 3 mm. to either side of the midline.

The disposition of the heat-sensitive area in the monkey is in close accordance with the location of the similar center previously outlined for the cat. Because the monkey is more closely related phylogenetically to man and because, like man, it sweats but does not pant, the results presented in this paper make it highly probable that the heat-sensitive area has the same location in man. This is in agreement with what little evidence is available from clinical studies.

The results are interpreted as identification of a reactive region which contains elements normally activated by the rising temperature of the blood when the animal is overheated, and in turn activating the mechanisms of heat-loss.

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THE ENDPLATE POTENTIAL DURING AND AFTER THE MUSCLE SPIKE POTENTIAL

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IN THE PRECEDING PAPER (Eccles and Kuffler, 1941) it was shown that a nerve impulse sets up in normal muscle an initial phase of endplate potential (e.p.p.), which is eventually submerged beneath the later spike potential. This initial phase may reach a height as small as 1 per cent or as large as 18 per cent of the peak-potential, but there is much less variation in its rate of rise, which is such that it would attain 30 to 42 per cent (exceptionally 50 per cent) of the peak-potential did the spike not supervene. Once the spike has arisen, the subsequent course of the e.p.p. is lost under the larger spike potential, and, as described in this paper, the following two indirect procedures must be used in determining the remainder of its time course.

(i) In the curarized frog's sartorius the course of the e.p.p. under a muscle spike has already been investigated by studying its interaction with an antidromic volley propagated from a distal point of direct muscle stimulation (Eccles, Katz and Kuffler, 1941b). When the antidromic volley arrives at the endplates synchronously with the setting up of the e.p.p. by a nerve volley, this potential is depressed to about 30 per cent of its height and greatly shortened in duration. If the assumption be made that the e.p.p. is identically affected by an antidromic muscle impulse and by an impulse initiated at the motor endplate, a similar investigation on normal muscle gives the course of the e.p.p. during and after the spike potential set up by a nerve volley. The antidromic impulse would have to be timed so that it arrived at the endplate zone at the instant of initiation of the muscle impulse there, and the course of the e.p.p. would be determined by subtracting the antidromic spike potential from the potential set up by the combined nerve and antidromic stimulation. However this method is only strictly applicable to the isolated single fibre nerve-muscle preparation, which is as yet unobtainable. In the present investigation the soleus strip preparation of the cat and the frog sartorius have been used as imperfect substitutes.

(ii) As shown previously (Eccles and Kuffler, 1941) subparalytic doses of curare diminish the initial phase of e.p.p. preceding the spike; at the same time this diminution also appears as a deficiency of the later part of the action potential at the endplate zone.

Besides determining the course of the e.p.p., this paper also describes excitability changes produced by that part of the e.p.p. which continues during and after the muscle spike potential, and discusses the way in which these effects are produced.

The experimental procedures have already been described (Eccles and O'Connor, 1939c; Eccles, Katz and Kuffler, 1941b). Care must be taken

that the stimulus directly exciting the muscle and setting up the antidromic volley does not also excite motor nerve fibres. With the cat's soleus the innervated strip was usually prepared by aseptic nerve dissection some four days previously, so as to allow time for degeneration of the nerves to the rest of the muscle. Stimulation of the nerve fibres innervating the strip was guarded against by placing the stimulating cathode on the strip at least 6 mm. away from the endplate focus, the anode being about 3 mm. further away.

A. Investigation by Antidromic Muscle Volleys (M)

1. *In cat.* In Fig. 1, the recovery of the mechanical response to a nerve volley is shown, following (i) an antidromic volley (crosses—*MN* series)

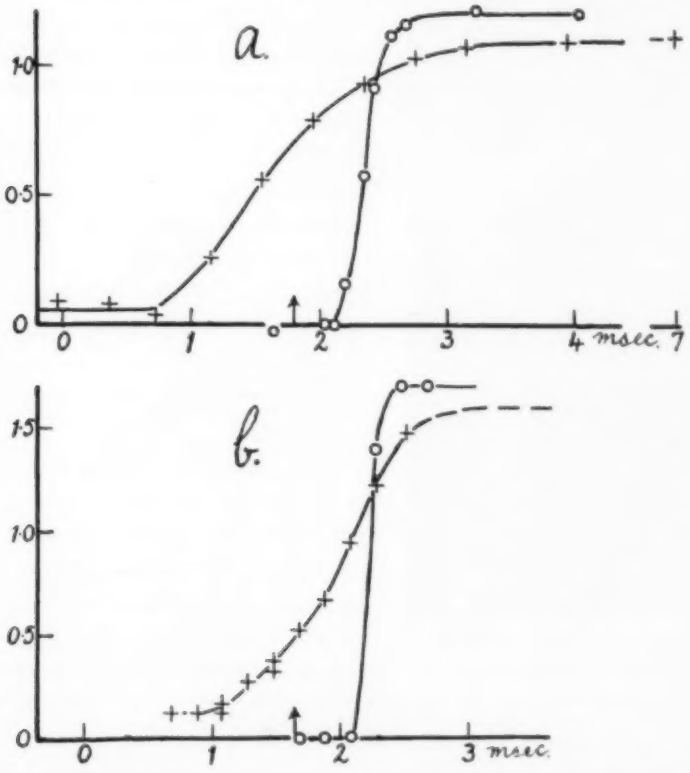

Fig. 1, *a and b.* Cat's soleus, two experiments. Contraction added by a nerve volley (*N*) set up at various times after a preceding (i) antidromic volley (*MN* series: crosses) or (ii) nerve volley (N_1N_2 series: circles). Ordinates, additional contraction expressed as a fraction of contraction evoked by *N* alone: abscissae for the N_1N_2 series are the stimulus intervals, and for the *MN* series the zero interval is chosen as that *MN* interval at which the *M* and *N* spike-peaks are simultaneously at the endplate zone (this spike-peak time indicated by arrow).

and (ii) a preceding nerve volley (circles—N_1N_2 series, cf. Eccles and O'Connor, 1939c, Fig. 10). In order to plot the two curves on a common time scale,

the zero for the *MN* series is chosen as that *MN* interval at which the *M* and *N* spike-peaks are simultaneous at the endplate zone (see arrow, Fig. 1). This zero for the *MN* series will be used throughout the paper. The two curves of Fig. 1a or b differ in two respects: (a) at the shortest *MN* intervals *N* gives a slight additional contraction, so forming a low initial plateau; and at long volley intervals the *MN* plateau is lower than the N_1N_2 plateau; (b) with the *MN* curve the rising phase begins earlier than with the N_1N_2 curve and is at least 1.5 msec. longer.

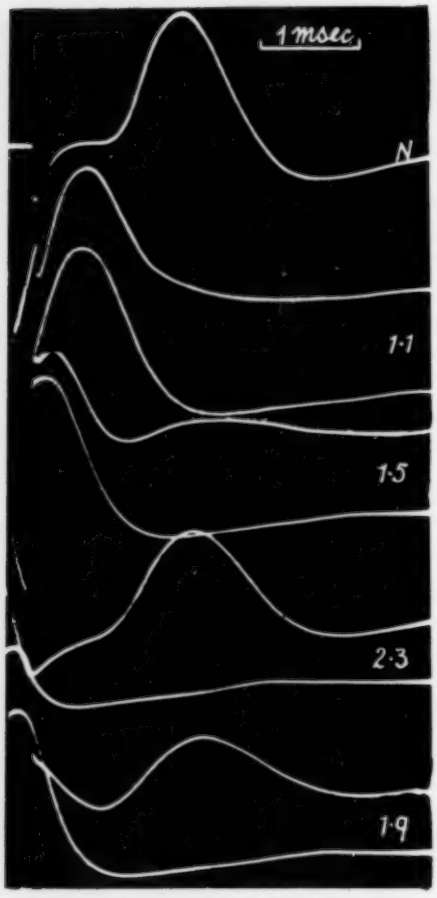

FIG. 2. Endplate zone of innervated strip, cat's soleus. Action potentials added by nerve volley (*N*) at the indicated intervals in milliseconds after the antidromic volley (*M*). The base line formed by the *M* response alone is just below the corresponding *MN* response. The *N* stimuli are fired at a constant position on the sweep, and at zero *MN* interval is chosen so that *M* and *N* spike-peaks are simultaneous. Top record, response to *N* alone, showing position of *N* stimulus for all *MN* records.

The difference (a) shows that the *M* stimulus has failed to excite some fibers of the innervated strip, these being fired off by *N*, even when simultaneous with *M*. The initial plateau height indicates that this failure occurred in about 6 and 12 per cent of the fibres in Fig. 1a and b respectively. Even with the most superficial and sharply defined of the innervated strips, it has been impossible to excite directly all their muscle fibres—in seven over 80 per cent were excited—in three over 90 per cent.

(b) The brief rising phase of the N_1N_2 curve indicates that there is very little scatter in the recovery times of the different muscle fibres; hence presumably the much longer duration of the *MN* rising phase is due to the asynchronism of arrival of the antidromic impulses at their respective endplates. This asynchronism would arise partly on account of differing conduction velocities in the individual muscle fibres, and partly of the differing conduction distances to the endplates, for there is a scatter over about 2 mm. of even a sharp endplate focus.

In Fig. 1 this asynchronism amounts to at least 1.5 msec., for which allowance must be made in interpreting a series of *MN* action potentials such as those of Fig. 2. In Fig. 3a the potentials added by *N* at various *MN* intervals have

been determined by subtraction. With the shortest *MN* intervals (0.7 and 1.1 msec.) similar potentials are added by the nerve volley, and progressively increasing spikes are shown as the *MN* interval lengthens to 1.5, 1.9 and

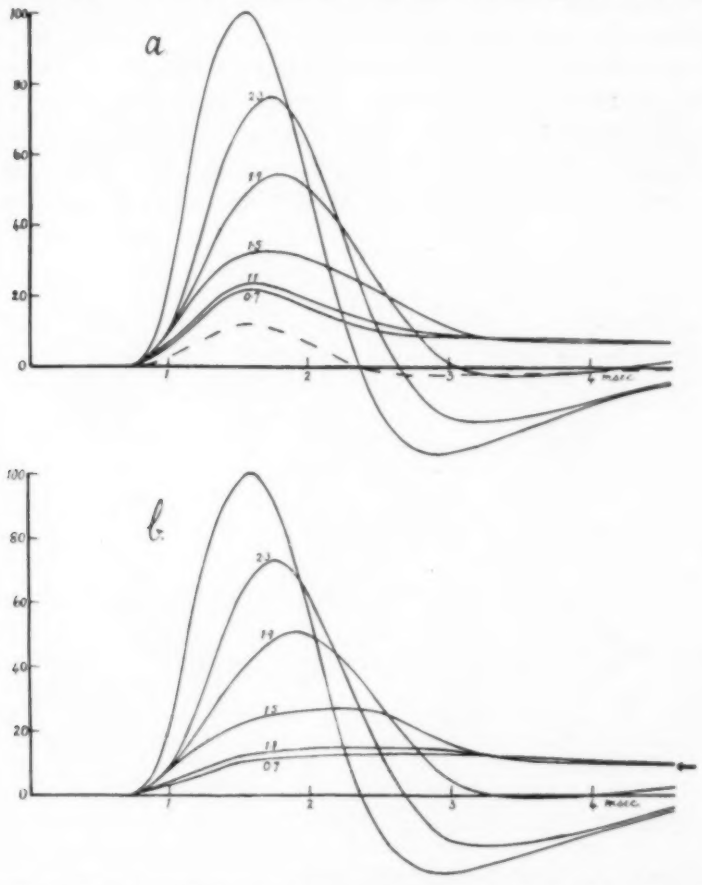

FIG. 3*a*. Potentials (percentages of normal peak-potential) added by *N* to *M* at the *MN* intervals shown in milliseconds on each curve (cf. fig. 2). The largest potential is response to *N* alone. The broken line is 12% of this *N* response and gives the action potential of the unoccluded fraction of innervated strip (see text). Zero time in this and all subsequent figs. is time of nerve stimulus.

FIG. 3*b*. As in fig. 3a, but the unoccluded fraction of potential has been subtracted from all curves (see text). The two arrows at extreme right of curves mark the heights of the e.p.p.'s with *MN* intervals of 0.2 and 0.4 msec. (see text).

2.3 msec. (cf. this same series in Fig. 1b and 2). The initial peaks in the 0.7 and 1.1 msec. responses indicate that the nerve volley sets up a small spike potential, and the contraction-interval curve (Fig. 1b) shows that this is due to that fraction of the innervated strip (12 per cent) not fired off antidromically. This unoccluded fraction of 12 per cent has been subtracted from all the curves of Fig. 3a to give the potentials set up by *N* in the occluded fraction (88 per cent) of the innervated strip (Fig. 3b). With

the 0.7 and 1.1 msec. intervals N adds a potential closely resembling in size and time course the e.p.p. added by N_2 at intervals of 1.7 and 2.1 msec. in the N_1N_2 series shown in Fig. 4. *Thus, when its spike response is prevented by a preceding antidromic volley, a nerve volley sets up an e.p.p., just as it does after the response to a preceding nerve volley.*

By the usual matching tests the "e.p.p. response-interval" curve has been plotted in Fig. 5a with circles for the N_2 responses (cf. Fig. 12 and 14, Eccles

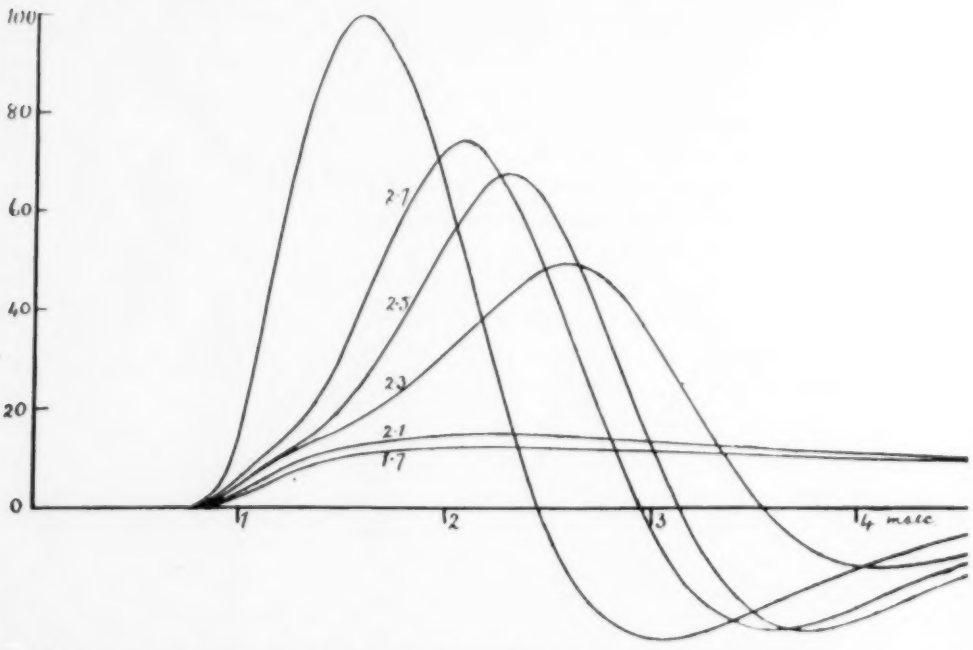

FIG. 4. Experiment of figs. 2 and 4. Potentials (percentages of normal peak-potential) added by N_2 to N_1 at volley intervals indicated in milliseconds on each curve. The largest potential is response to N alone. At N_1N_2 intervals of 1.7 and 2.1 msec. N_2 sets up an e.p.p. only, and an initial e.p.p. step is seen at the three longer intervals.

and Kuffler, 1941). The two upright crosses show the points for the e.p.p.'s set up by N at the MN intervals of 0.7 and 1.1 msec. (cf. Fig. 3b). With longer MN intervals the early origin of the spike prevents any estimate of the endplate potential in Fig. 3b. Moreover, on account of the asynchronism, an antidromic volley, with MN intervals less than 0.7 msec., would fail to reach the last endplates before N had caused them to discharge impulses. This makes it impossible to determine the initial part of the N response by the subtraction analysis. However, that part of the N response beyond the range of such spike interference can be determined and is found to have a time course similar to the e.p.p. set up by N at the 0.7 and 1.1 msec. intervals and to be little if any smaller (cf. arrows to right of Fig. 3b). Assuming that the time courses are also similar over the earlier unanalysable parts, the

maximum potentials may be calculated and are plotted as the two oblique crosses in Fig. 5a.

Figure 5b gives a more complete MN and N_1N_2 series plotted as in Fig. 5a (the N_1N_2 responses have been plotted in Fig. 12, Eccles and Kuffler, 1941). At zero interval, *i.e.*, with simultaneous M and N spikes, N sets up an e.p.p. about 11 per cent of the peak-potential. In three other experiments (cf. Fig. 5a) this e.p.p. has also been about 10 per cent of the peak-potential (respective values: 11, 8.5, 9.5). In our other antidromic experiments an e.p.p. of this order has also been observed for short MN response intervals. *It may, therefore, be concluded that at the endplate zone an e.p.p. of about 10 per cent of the peak-potential is submerged beneath the spike potential set up by a nerve volley.*

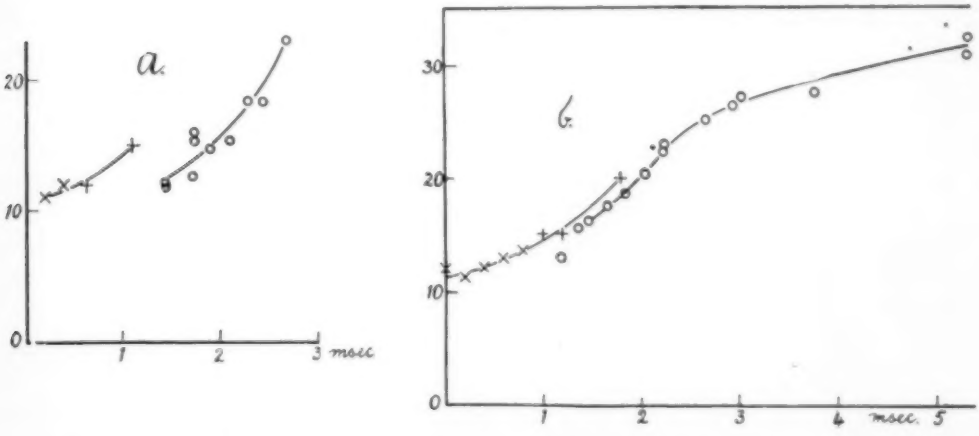

FIG. 5. Ordinates, size of e.p.p. (as percentages of peak-potential) set up by a nerve volley after a previous nerve volley (circles) or antidromic volley (crosses): abscissae, intervals in milliseconds measured as in fig. 1 for N_1N_2 and MN series.

a, experiment partly shown in figs. 1b, 2, 3 and 4.

b, experiment shown in figs. 10, 11 and 12 of previous paper (Eccles & Kuffler, 1941).

Normally the rate of rise of the e.p.p. set up by a nerve volley is such that it would attain 30 to 40 per cent of the peak-potential did the spike not supervene (Eccles and Kuffler, 1941). During the refractory period set up by an antidromic volley this maximum is diminished to about 10 per cent of the peak-potential, *i.e.*, to about 30 per cent of its normal value. It is of interest that the antidromic volley has an almost identical effect on the e.p.p. in completely curarized muscle (Eccles, Katz and Kuffler, 1941b).

The MN series of Fig. 5a and b show the increasing e.p.p. set up by N as the MN interval lengthens, *i.e.*, as tested by the e.p.p. response, there is a progressive recovery from the refractoriness. In both figures there is a suggestion that with the N_1N_2 series, the e.p.p. set up by N_2 recovers along a slightly lower curve; however, these figures certainly indicate that the refractoriness of the muscle is largely responsible for the diminished e.p.p.'s

set up by N_2 at short N_1N_2 intervals. The closeness of the N_1N_2 and MN curves in Fig. 5b also suggests that the preceding N_1 produces no large diminution in the exciting power of N_2, until the N_1N_2 interval is less than 1.5 msec. This is also indicated by the e.p.p.'s set up by N_2 at various intervals in completely curarized muscle (cf. Eccles, Katz and Kuffler, 1941b, Fig. 17a).

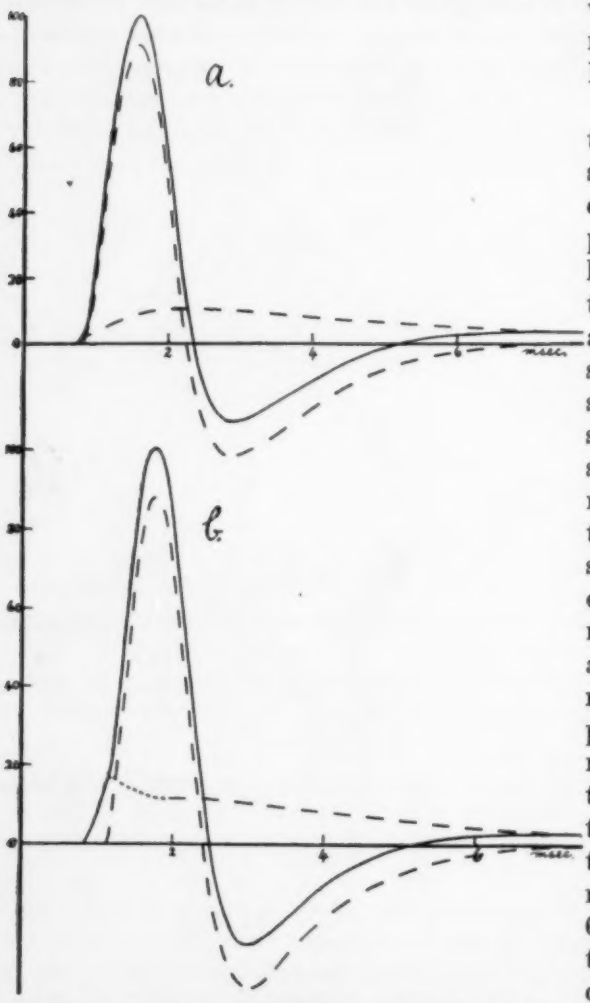

FIG. 6. Continuous line shows action potential set up at endplate zone by a nerve volley. Lower broken line shows e.p.p. component of this potential as determined by antidromic experiments. Upper broken line shows spike potential as determined by subtracting e.p.p. from total potential. Ordinates, potentials as percentages of peak potential: abscissae, time after nerve stimulus.
a, experiment of figs. 1b, 2, 3, 4, 5a.
b, experiment of fig. 5b.

The antidromic investigation has given the approximate size and time course of the e.p.p. which underlies the spike potential set up by a nerve volley; hence the total action potential set up by a nerve volley at the endplate zone may be separated into its component spike and e.p.p. Fig. 6a shows such an analysis for the simple-spike potential set up by a nerve volley in Fig. 2. When there is a large initial e.p.p. step (cf. Fig. 1, 4 and 10, Eccles and Kuffler, 1941), the membrane breakdown associated with the spike presumably results in a diminution of this potential, for the initial step may actually be greater than the maximum e.p.p. added on top of the spike. Some indication of the speed of this diminution (see dotted line in Fig. 6b) is given by the action of the antidromic volley on the decay of the e.p.p. in curarized muscle (cf. Eccles, Katz and Kuffler, 1941b).

2. *In frog's sartorius.* Frog's sartorius is in some respects more suitable for investigating the interaction between M and N, especially as complete occlusion can be achieved by maximal stimulation of all the muscle fibres.

The e.p.p.'s added by the testing nerve volley are determined by subtraction as in Fig. 3a and 4, and *MN* intervals are measured as above so as to be comparable with the N_1N_2 intervals. With similar short *MN* and N_1N_2 intervals (1.0–1.5 msec., 23°C.) the added e.p.p.'s do not differ significantly (cf. Fig. 5b). However, as the *MN* interval was still further shortened, the e.p.p. was much diminished and decayed more rapidly. With zero *MN* interval it was only about 40–50 per cent of that set up by N_2 at N_1N_2 intervals of 1.0–1.5 msec., and little survived the antidromic spike potential. Thus it may be concluded that the frog's sartorius resembles the cat's soleus in that an e.p.p. is submerged beneath the spike potential set up by a nerve volley, but it appears to be smaller and very little survives the spike potential.

B. Investigation by Curarization

1. Effect on action potential set up by a single nerve volley

Subparalytic curarization diminishes the rate of rise of that part of the e.p.p. which precedes the spike origin (Eccles and Kuffler, 1941). At the same time there is always a diminution in the negativity at the endplate zone for some time after the spike potential (cf. Fig. 13a), and in Fig. 7a and b, curve *ii*, the time course of this diminution has been determined as the difference between the potentials set up at the endplate zone by a single nerve volley before and after subparalytic curarization. Beyond 6 msec. the time course of decay resembles that for the e.p.p. set up by a second nerve volley in the normal muscle (curve *i*), and at shorter intervals the deviation is explicable by the delay of the spike potential produced by subparalytic curarization (cf. Eccles and Kuffler, 1941, Fig. 4). Hence this deficient negativity is due to a diminution by curare of that part of the e.p.p. which normally outlasts the spike potential set up by a single nerve volley.

In normal muscle a deficiency with a similar time course is shown for the response to a second nerve volley at intervals from 20 to 200 msec. after the first volley. Usually this deficiency is about 20 per cent of the e.p.p. set up by an early second nerve volley, but in curve *iii*, Fig. 7b, it is as much as 50 per cent (curve *i*). Curves *iv*, Fig. 7a and b, show that the depression of the second volley and subparalytic curarization are cumulative in producing a deficiency of e.p.p.

2. Effect on action potential set up by an early second nerve volley

Figure 8a shows typically that subparalytic curarization not only diminishes the e.p.p. added by an early second nerve volley, but also quickens its rate of decay. Normally (curve *i*) this rate is slower than with the pure e.p.p. of completely curarized muscle (curve *iv*), but, with a depth of curarization just less than partially paralytic (curve *iii*), there is considerably faster decay than in complete curarization. However, plotting semi-logarithmically (Fig. 8b) shows that with all depths of curarization the later parts of the e.p.p.'s (from about one half decay onwards) are approximately parallel straight lines, *i.e.*, all have practically the same rate of exponential

decay. With curarized muscle this phase of decay is due to passive dissipation of membrane potential (Eccles, Katz and Kuffler, 1941b); hence the present experiments show that curare has no appreciable action on the electric constants of the muscle membrane. The initial slow rate of decay with normal muscle must be due to the persistent action of some depolarizing agent—

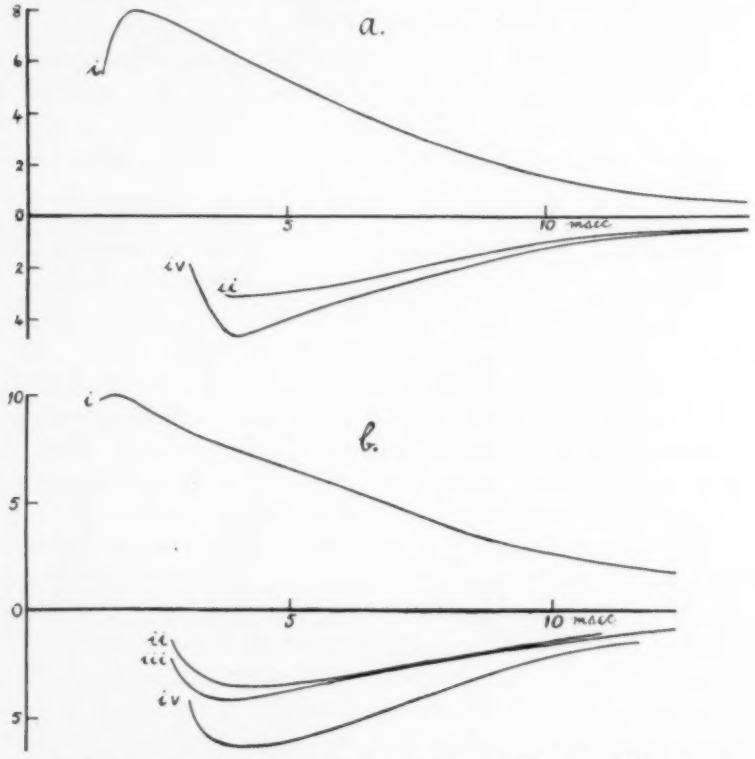

FIG. 7, *a and b* show two experiments on cat's soleus with recording electrode on end-plate zone of innervated strip. Ordinates, percentages of peak-potential: abscissae, time in milliseconds from stimulus. Curve i is the e.p.p. added by a second nerve volley 1.6 msec. after the first, zero being time of N_2 stimulus. Continuous line below base line is deficiency which subparalytic curarization produces in action potential set up by a single nerve volley. Curve ii shows deficiency (relative to normal single response) of action potential set up a second nerve volley 24 msec. after first. Curve iv shows deficiency (relative to normal single response) of action potential set up in subparalytically curarized muscle by second nerve volley 24 msec. after first. Zero time for curves below base line is time of nerve stimulus.

presumably that acting initially and producing the e.p.p. Thus subparalytic curarization not only diminishes the effect produced by this depolarizing agent (cf. Eccles and Kuffler, 1941, section A1), but it also shortens its duration of action. Such shortening of action will not account for an initial deacy faster than with the completely curarized response (cf. curves *iii* and *iv*, Fig. 8), for, if anything, the action should then be still shorter. However, it has been shown that, on account of diminished membrane impedance, the

e.p.p. decays much more rapidly in refractory muscle (Eccles, Katz and Kuffler, 1941), and presumably this is the explanation of the rapid initial rates of decay with the deepest subparalytic curarizations. Thus we have two opposing factors normally modifying the decay of the e.p.p. set up by an early second nerve volley: (i) the refractoriness resulting from the muscle

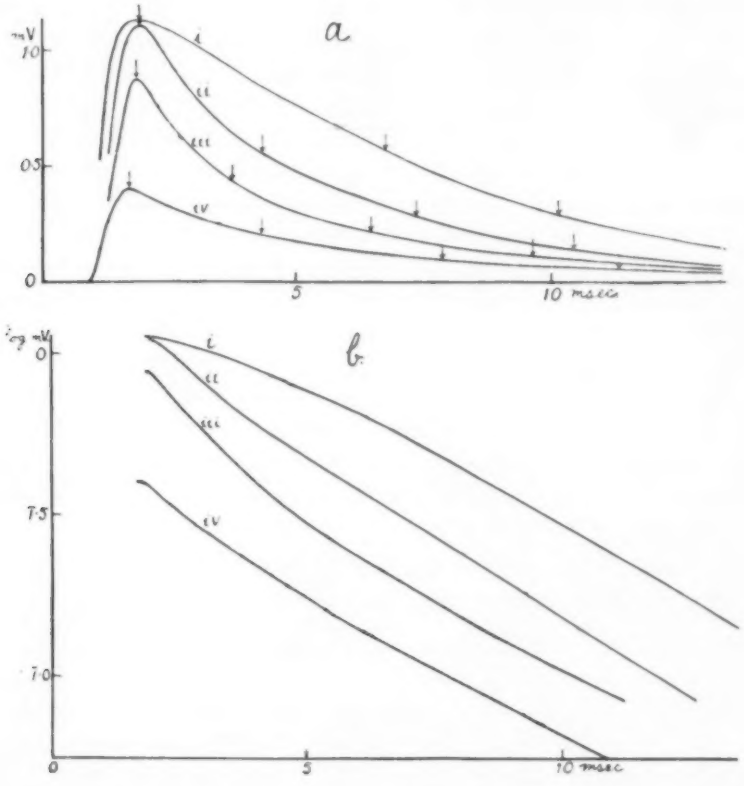

FIG. 8*a*. Cat's soleus, endplate zone of innervated strip. Ordinates, potentials in mV.: abscissae, tine in milliseconds from nerve stimulus. Upper three curves show e.p.p. set up by N_2 1.6 msec. after N_1. i, normal muscle; ii and iii, subparalytic curarization; iv, e.p.p. set up by a single nerve volley in complete curarization. Arrows mark summits and times of half, quarter and eighth decay of e.p.p.

FIG. 8*b*. As in fig. 8a, but ordinates are logarithms of potentials. Only decaying phases of e.p.p.'s are plotted.

spike response to the first nerve volley tends to make the rate of decay greater during the first 6 msec., and (ii) the depolarizing agent responsible for setting up the e.p.p. continues to exert its active depolarizing effect for about 6 msec. This latter action is normally more powerful than the former, so initially the e.p.p. decays more slowly than in completely curarized muscle; with subparalytic curarization the situation is reversed.

The antidromic experiments (section A) have shown that the e.p.p.'s

set up by a single nerve volley and by an early second nerve volley decay similarly (cf. Fig. 3b and 4); hence the above two factors also presumably condition the decay of the e.p.p. set up by a single nerve volley in normal muscle. The persistent action exerted on the muscle membrane by the depolarizing agent accounts for the normal e.p.p. outlasting the spike potential in much the same way as the e.p.p. set up by an early second nerve volley. On the other hand it has been shown that in curarized muscle the depolarizing action is very transient and practically no e.p.p. then outlasts the rapid period of decay that occurs during a muscle spike (Eccles, Katz and Kuffler, 1941b). Even with subparalytic curarization, curve *iii*, Fig. 8a, suggests that, in the response to a *single* nerve volley, very little e.p.p. would outlast the muscle spike. Therefore subtraction of such a subparalytic potential from the normal response to a single nerve volley should give nearly the full normal e.p.p. for the region after the spike. Thus, for example, in Fig. 7a the e.p.p. of curve *ii* is 65 per cent of the e.p.p. set up by an early second nerve volley (curve *i*), and curve *iv* is actually 80 per cent of curve *i*. Therefore in its later part, at least, the e.p.p. set up by a single nerve volley must be at least 80 per cent of that set up by an early second nerve volley, a result in agreement with the antidromic experiments (cf. Fig. 5a and b).

Normally the frog's sartorius resembles the subparalytically curarized cat's soleus, in that very little e.p.p. survives the spike potential set up by a single nerve volley (Section A2); this suggests that the depolarizing action is briefer.

C. Effects Produced by the Action of the Endplate Potential During and After the Muscle Spike

In sections A and B it has been shown that part of the e.p.p. set up by a nerve volley persists during and after the muscle spike also set up by this volley. It will be seen (section 1) that this concurrent e.p.p. lengthens the minimum interval at which a later "testing" nerve volley sets up a muscle spike—"the least spike-interval," *i.e.*, the e.p.p. delays recovery from the muscle's refractory period. In addition it will be seen (Section 2) that the concurrent e.p.p. raises the threshold e.p.p. at which the spike is initiated.

1. Lengthening of least spike-interval by the endplate potential

The following five investigations are designed to give varying sizes and time courses of the e.p.p. during and after a muscle spike, and the effect of this e.p.p. is tested by the response to a later nerve volley. As this testing nerve volley is always more than 1.5 msec. after the immediately preceding nerve volley, no serious error is introduced by neglecting any diminution which this preceding volley may produce in its excitatory power (cf. Section A1). With short volley intervals the preceding nerve volley lengthens the latency of the e.p.p. set up by the testing nerve volley, but with intervals beyond 1.5 msec. this is no more than 0.1 msec. (cf. Fig. 22, Eccles and O'Connor, 1939c) and may be neglected when considering the much greater lengthenings of spike latency.

a. Comparison of MN with N_1N_2 series. In Fig. 9 the ordinates are the latent periods of the muscle spike-peaks set up by the testing nerve stimulus, and abscissae are the time intervals between the *M* and *N* or the N_1 and N_2 stimuli brought to a common time scale as in Fig. 1 above. As the testing *N* stimulus is moved closer to the initial spike-peak (shown by arrow in Fig. 9) there is a lengthening of the spike's latent period with both the N_1N_2 and *MN* series. With the *MN* series the curve eventually rises at 45°, showing that the latent period is lengthened by an amount equal to the shortening of the *MN* interval, *i.e.*, the intervals between the *M* and *N* spike-peaks reach a constant value, which the intersection of the 45° line with the base line shows to be 2.15 msec. The 3 points for the N_1N_2 series in Fig. 9 give the least spike-interval for the N_1N_2 series as 3.15 msec., again by the intersection of the 45° line with the base line. Thus Fig. 9 shows that the least spike-interval is lengthened from 2.15 msec. to 3.15 msec. when an e.p.p. is present during the refractory period. Table 1, column 4, shows the lengthenings of least spike-intervals in the 8 experiments in which this investigation has been carried out, the average being 0.83 msec. with extremes of 0.6 and 1.1 msec.

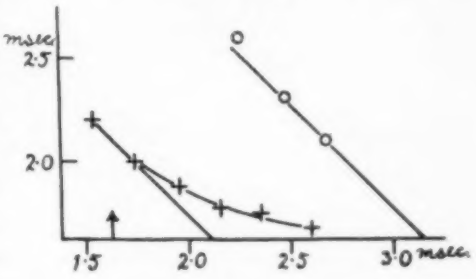

FIG. 9. Cat's soleus, endplate zone. Ordinates, latent periods of muscle spike-peaks set up by testing nerve stimulus at various intervals: abscissae are stimulus intervals with the N_1N_2 series (circles—cf. fig. 4), and with the *MN* series (crosses—partly shown in figs. 2 and 3); zero interval is chosen so that *M* and N_1 spikes synchronize at the arrow (cf. fig. 1). Base line is drawn at normal latent period for spike set up by *N*, hence intersections with 45° lines give the least spike-intervals for the *MN* and N_1N_2 series.

It has been observed that sometimes there is supernormal conduction of the earliest muscle impulses set up by a second nerve volley (Eccles and O'Connor, 1939c, p. 95). This suggests that recovery from refractory period is slower at the endplate zone than elsewhere in the muscle, an effect attributable at least partly to the e.p.p.

b. Comparison of MnN series with the MN and N_1N_2 series. This relationship of e.p.p. to lengthening of the least spike interval has been further investigated in two antidromic experiments by interpolating a conditioning nerve volley (*n*) between the antidromic volley, *M*, and the testing nerve volley, *N*. In Fig. 10 several such series of observations for different *Mn* intervals are plotted as in Fig. 9, together with the *MN* and N_1N_2 curves. When *n* is timed so that its spike response would approximately coincide with the antidromic spike, it lengthens the spike-intervals so that the recovery curve approximates to that for N_1N_2 (cf. the *M* 0.1 msec. *n* curve in Fig. 10), as would be expected if *n* added the same e.p.p. to the antidromic spike that it would normally add to its own muscle spike response. This supports the original assumption at the beginning of this paper, which

Table 1. Table showing in columns 2, 3, 6 and 8 the least spike-intervals in milliseconds for the various series of stimuli described in Section C1

Experiment	Section C1a: *MN*, N_1N_2			Section C1b: *MnN*		Section C1c: N_1nN_2		
	MN least spike intervals	N_1N_2 least spike intervals	Lengthening: column 3 − column 2	*Mn* intervals	Least spike intervals	N_1n intervals	Least spike intervals	Average lengthening by *n*: column 8 − column 3
1	2	3	4	5	6	7	8	9
2/11/38		2.6				0.8 1.2 1.4 1.6 1.8 2.0	3.65 3.65 3.7 3.8 3.4 3.5	1.0
8/11/38		2.8				1.6 1.9	4.3 3.8	1.15
11/11/38		3.0				1.0 1.4 1.8	3.85 3.85 4.25	1.0
24/ 2/39		2.6				1.0 1.4 1.8	4.0 4.2 4.1	1.5
2/ 3/39		3.0				1.1 1.7	4.3 4.0	1.15
4/ 8/39		2.55				1.15	3.8	1.25
18/ 8/39	2.1	3.0	0.9	0 0.15 0.3	3.0 3.3 3.6			
12/ 9/39	2.1	2.8	0.7					
15/ 9/39	2.0	3.1	1.1					
27/10/39	2.8	3.4	0.6	0.1 0.45 0.95 1.3 1.7	3.3 3.6 4.05 4.35 4.6	1.2 1.9	5.2 5.4	1.9
4/12/39	2.2	2.85	0.65					
18/ 4/40	2.2	3.0	0.8					
4/ 7/40	2.15	3.15	1.0					
20/ 9/40	2.2	3.05	0.85					
Average lengthenings			0.83					1.28

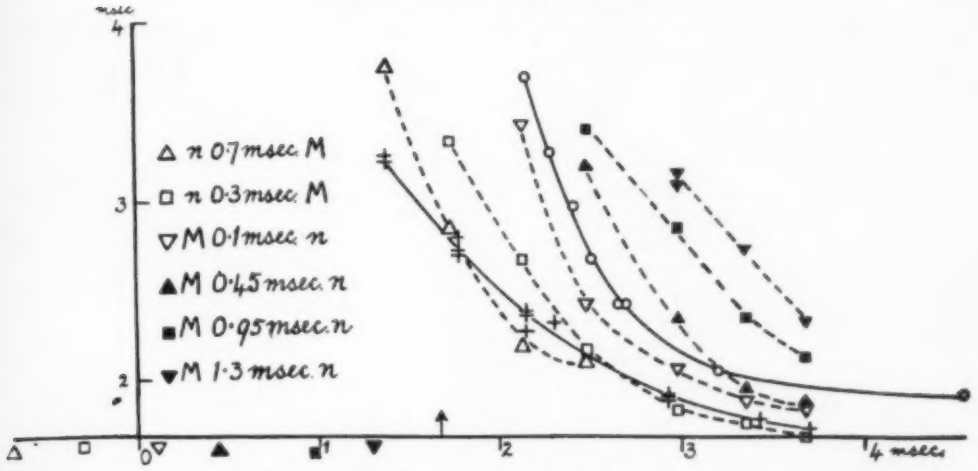

FIG. 10. *MnN* series. *MN* (crosses) and N_1N_2 (open circles) plotted as in fig. 9 (another experiment), but 45° lines not drawn. In addition a conditioning nerve volley, *n*, has been set up at six different intervals before or after *M*, and the corresponding six series of points with their broken-line curves give the latencies of the spike-peaks set up by the later testing nerve volley. The *Mn* intervals are shown by different symbols and the time of the *n* stimulus relative to the *M* (at zero time) is indicated by the corresponding symbol below the base line. The arrow shows time of *M* spike-peak.

formed the basis of the determination of the e.p.p. set up normally by a single nerve volley (Section A). With *n* later relative to *M*, *i.e.* with *n* setting up its e.p.p. some time after the beginning of the antidromic spike, Fig. 10 shows that the spike-intervals are still further lengthened. Thus, with the interpolated e.p.p. beginning 0.45, 0.95 and 1.3 msec. after the antidromic spike, the respective least spike-intervals are lengthened by 0.8, 1.25 and 1.55, as against a lengthening of only 0.6 msec. for the N_1N_2

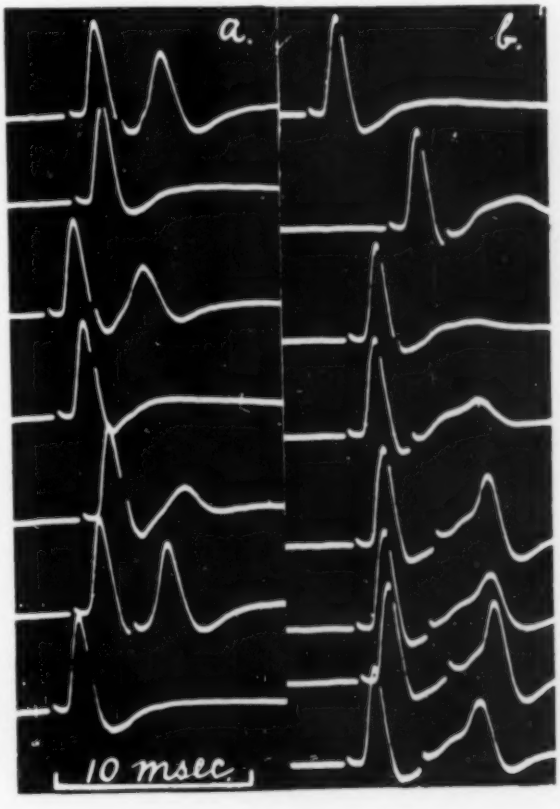

FIG. 11. Same experiment as fig. 10.

a. N_1N_2 series: intervals from top down in milliseconds: 2.8, 1.8, 2.55, 2.15, 2.4, 3.2; lowest record N_1 alone.

b. N_1nN_2 series: N_1n constant at 1.9 msec: top record, N_1n: remainder, N_1nN_2 series at N_1N_2 intervals from top down in milliseconds: 3.1, 2.7, 3.5, 4.3, 3.9, 4.7, 4.05.

curve. Two factors would give this effect: (i) the later the interpolated e.p.p., the larger it would be (Fig. 5); (ii) the later the e.p.p. the longer it would survive, and hence the more effective it would be in prolonging the refractory period.

When the *n* volley is so early relative to *M* that it sets up its muscle spike before *M*, the latent period curve should be identical with the N_1N_2 curve, for the antidromic volley (*M*) is blocked from reaching the endplates. With the plotting of Fig. 10 such curves would be N_1N_2 curves shifted to the left by the time the *n* spike *precedes* the *M* spike and this is seen for the *n* 0.7 msec. M curve, but with the *n* 0.3 msec. *M* curve, the shift is 0.1 msec. more than would be expected, and, with *M* 0.1 msec. *n*, the curve is 0.2 msec. earlier.

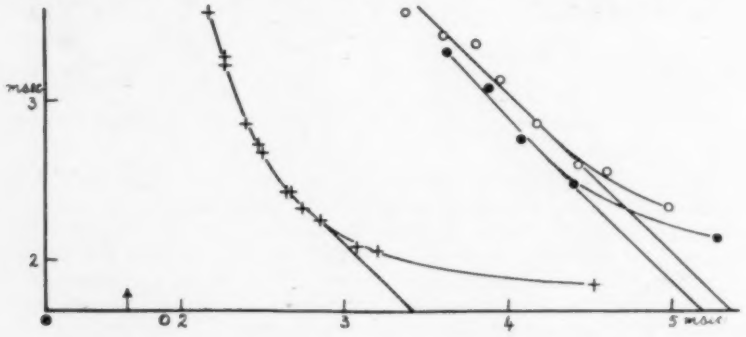

FIG. 12. Plotting as in fig. 9 of results partly shown in fig. 11. Crosses show N_1N_2 curve (cf. N_1N_2 curve open circles, of fig. 10). Open and closed circles show effect on spike latent periods of conditioning volley, *n*, 1.9 msec. (fig. 11b) and 1.2 msec. after N_1. Positions of *n* relative to N_1 shown by closed and open circles below base line as in fig. 10. N_1 stimulus is at zero on scale and arrow shows time of its spike-peak.

Possibly this shift is partly due to the muscle spike being speeded up by the concurrence of two excitatory mechanisms—the eddy currents preceding the propagating antidromic volley and the depolarizing effect of the neuromuscular transmitter. In curarized muscle the e.p.p. was similarly observed to produce a slight speeding (0.1 to 0.2 msec.) of the antidromic volley (Eccles, Katz & Kuffler, 1941 b.).

c. Comparison of N_1nN_2 with N_1N_2. An early second nerve volley (*n*) adds its e.p.p. to that of the first volley (N_1) without setting up a spike potential (cf. Fig. 4), and, correspondingly, comparison of Figs. 11a and b shows that there is a lengthening of the least spike-interval as tested by a third nerve volley (N_2). This is the "brief depressant action" described by Eccles and O'Connor (1939a). In Fig. 12 the interpolated nerve volley, *n*, at 1.2 or 1.9 msec. after N_1 lengthens the least spike-interval as much as 2 msec. The above factors *i* and *ii* of section *b* might account for this lengthening being so much greater than the 0.6 msec. lengthening produced by the e.p.p. of a single nerve volley acting synchronously with the muscle spike (comparison of N_1N_2 and *MN* curves in Fig. 10).

However Columns 7 and 8, Table I, show that there are limits to the action of these factors, for there is no significant relationship between the N_1n interval and the lengthening of the least spike-interval. But, for very short N_1n intervals, *n* sets up an e.p.p. at a constant interval of about 1.4 msec. after the N_1 response (Eccles and O'Connor, 1939c, p. 77); hence these observations of Table 1 are not inconsistent with those in columns 5 and 6 (*MnN* series, cf. Fig. 10).

d. N_1N_2 before and after subparalytic curarization. The superimposed records of Fig. 13a show the diminution which a subparalytic dose of curare produces in the e.p.p. that survives the spike (cf. Section B1). Associated with this diminution there is a shortening of the least spike-interval (Fig. 13c and d), the spike arising much earlier on the course of the e.p.p. set up by N_2. The diminution of e.p.p. produced by subparalytic curare has always (seven experiments) been associated with a diminution in the least spike-interval.

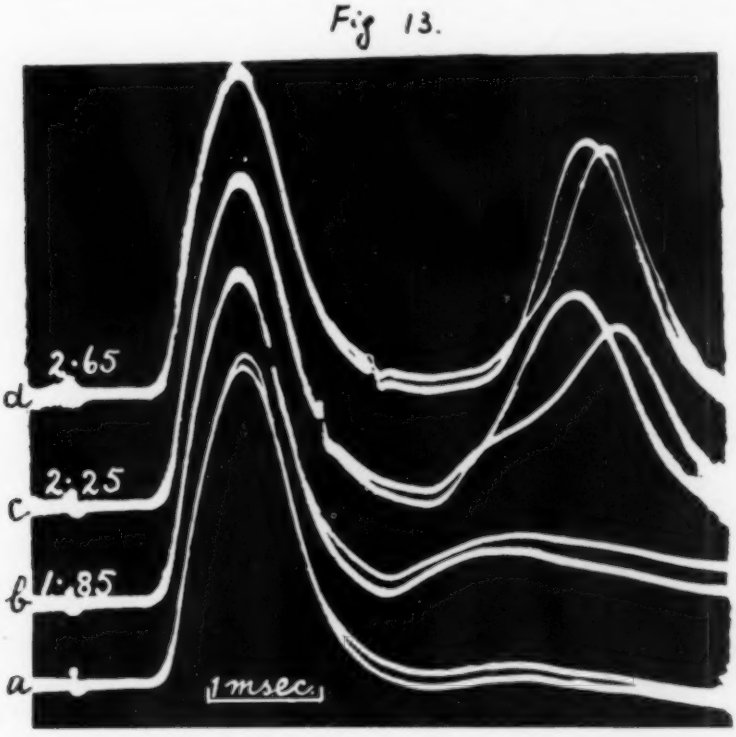

FIG. 13. Endplate zone, cat soleus; a, b, c and d each show superimposed records of action potentials set up before and after subparalytic curarization: a, single nerve volley; b, c and d, two nerve volleys at intervals indicated in milliseconds. After the spike curare is seen to produce a deficiency of the action potential set up by a single nerve volley (see a, and also b, c and d before response to 2nd volley).

This diminution has varied from 0.3 to 0.5 msec., and so is about half the difference between the N_1N_2 and *MN* least spike-intervals (Table 1, column 4). Thus in subparalytic curarization there is good agreement between this diminution of least spike-interval and the diminution of e.p.p., which is also about halved (column 3, Table 1, Eccles and Kuffler, 1941).

e. N_1N_2 before and after eserinization. Eserine increases and prolongs the e.p.p. set up by a single nerve volley (Eccles and O'Connor, 1939b; Eccles, Katz and Kuffler, 1941a), and correspondingly there is a lengthening of the least spike-interval. With increasing dosage the e.p.p. and the least spike-interval increase *pari passu*, until a maximum effect is attained with about

0.5 mg. per kg. Table 2 shows that this maximum lengthening of least spike interval averaged 2.0 msec. In four of these experiments eserine was shown (cf. Table 2, *MN* series) to have no significant action on the least spike-interval when the testing nerve volley follows an antidromic volley, *i.e.* it has no action on the true refractory period of the muscle. The action of eserine will be fully described in a later paper.

Table 2. Table showing least spike-intervals in milliseconds for MN and N_1N_2 series normally and after a dose of eserine sufficient to give the maximum lengthening with the N_1N_2 series (cf. Section C1e)

Experiment	*MN* series		N_1N_2 series		
	Normally	After eserine	Normally	After eserine	Lenthening
4/10/38			3.1	5.4	2.3
25/10/38			3.3	5.6	2.3
24/ 7/39			3.0	6.0	3.0
4/ 8/39			2.55	4.3	1.75
18/ 8/39	2.1	2.15	3.0	5.0	2.0
12/ 9/39	2.1	2.1	2.8	4.2	1.4
15/ 9/39	2.0	2.2	3.1	4.6	1.5
11/10/39			3.2	5.1	1.9
27/10/39	2.8	2.85	3.4	5.1	1.7

Discussion

The above evidence shows that the muscle's refractory period is lengthened by concurrent e.p.p., and, when this is large, the least spike-interval may even be doubled (cf. three experiments of Table 2). Now e.p.p. is a cathodal polarization of the junctional region of the muscle (Eccles, Katz and Kuffler, 1941b); hence these observations are related to those of Blair and Erlanger (1933, p. 546), who find that a nerve's refractory period is lengthened by a brief intercurrent cathodal polarization (cf. also Bugnard and Hill, 1935).

2. THE THRESHOLD E.P.P. FOR INITIATING A SPIKE

When the normal action potential has a large initial e.p.p. step (12–18 per cent of the peak-potential), it is probable that this e.p.p. is solely responsible for setting up the muscle impulse, *i.e.* the spike potential (Eccles and Kuffler, 1941). The time course of the e.p.p. during and after the spike can be determined as in Section A (cf. Fig. 6); hence it is possible to construct the time courses of the total e.p.p. set up by two nerve volleys at various short intervals apart. This has been done in Fig. 14a for a double-step experiment in which the spike arose when the initial e.p.p. was 18 per cent of the peak-potential. The e.p.p. set up by each second nerve volley (N_2) is added on to the background e.p.p. of N_1, and the point of spike origin is ringed by a circle. The summed e.p.p. curve is thereafter concealed under the spike and so can be continued no further. Through these spike origins of Fig. 14a a curve may be drawn, which gives the threshold e.p.p. as determined by N_2

at various times after the muscle's response to N_1. At the shortest interval this threshold is more than 50 per cent above the normal threshold, and with lengthening N_1N_2 intervals it declines at first rapidly, then more slowly, being still considerably above threshold with N_2 6 msec. after N_1.

However the responses at still shorter N_1N_2 intervals (Fig. 14b) show that N_2 does not merely test the e.p.p. threshold at various times after N_1, but

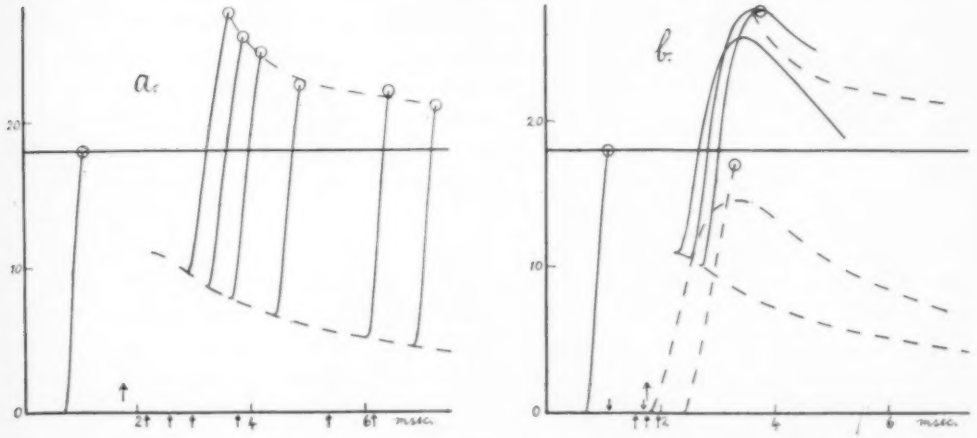

FIG. 14*a*. Endplate zone, cat soleus, N_1N_2 series (experiment of figs. 10 & 11, Eccles & Kuffler, 1941). Ordinates, e.p.p. as percentage of peak-potential; abscissae, time after N_1 stimulus. To left the initial e.p.p. is shown rising steeply and setting up a spike at the normal threshold e.p.p. (18%). Arrow pointing upwards above base line gives time of spike-peak. Lower broken line gives subsequent course of N_1 e.p.p. as determined by antidromic experiment. On top of this is shown the e.p.p. added by N_2 at the six N_1N_2 intervals shown by arrows below the base line. For each the e.p.p. begins about 0.7 msec. later and rises to set up a spike at point ringed by circle. Upper broken line shows e.p.p. threshold curve.

FIG. 14*b*. As in fig. 14a, but for the three shorter N_1N_2 intervals shown by arrows below base line. The continuous lines show the corresponding e.p.p.'s added on to the background N_1 e.p.p., a spike being set up only at longest N_1N_2 interval. Arrows pointing downwards above base line show two positions of N stimulus in corresponding MN series, the M spike-peak being synchronous (at large arrow) with that for N_1 of N_1N_2 series (cf. fig. 1). The two broken lines beginning about 0.7 msec. later than the N stimuli show courses of e.p.p. set up by N, the spike origin with M 1.85 msec. N being ringed by the circle (further description in text).

it also conditions this threshold to some extent. Thus with N_1 1.75 msec. N_2 the summed e.p.p. goes well above the threshold curve of Fig. 14a, and yet sets up no spike, while at N_1 1.95 msec. N_2, a spike is not initiated till about 0.15 msec. after the summed e.p.p. has crossed the threshold curve, *i.e.* shortening the N_1N_2 interval from 2.15 msec. to 1.95 msec. lengthens the spike-interval by 0.15 msec. This effect is always observed, and has already been described as an example of the Lucas C type of curve (Eccles and O'Connor, 1939c, p. 79). Presumably N_2 raises the e.p.p. threshold in this way on account of the e.p.p. which it adds to the background e.p.p. of N_1. With the longer volley intervals Fig. 14a shows that the e.p.p. set up by N_2

rises so steeply that it sets up a spike before it has much effect in delaying recovery or raising the e.p.p. threshold. But, as the volley interval is shortened, the e.p.p. of N_2 rises less steeply (cf. Fig. 11 and 13, Eccles and Kuffler, 1941), ane so takes longer to initiate a spike; hence there is a longer time in which it acts to raise the e.p.p. threshold. Thus, as the N_1N_2 interval is shortened, the threshold e.p.p. curve of Fig. 14 is raised progressively more by the e.p.p. of N_2, and finally, at N_1 1.75 msec. N_2, the rate of rise of e.p.p. set up by N_2 is so small that the e.p.p. threshold is raised above the whole course of the summed e.p.p. and a spike is never initiated. In the double nature of its action the e.p.p. thus resembles the analogous local potential set up in nerve by a cathodal stimulus (cf. Hill, 1936); both tend to initiate an impulse and at the same time to raise the threshold at which an impulse is initiated. This accommodation process appears to be particularly intense during the refractory period.

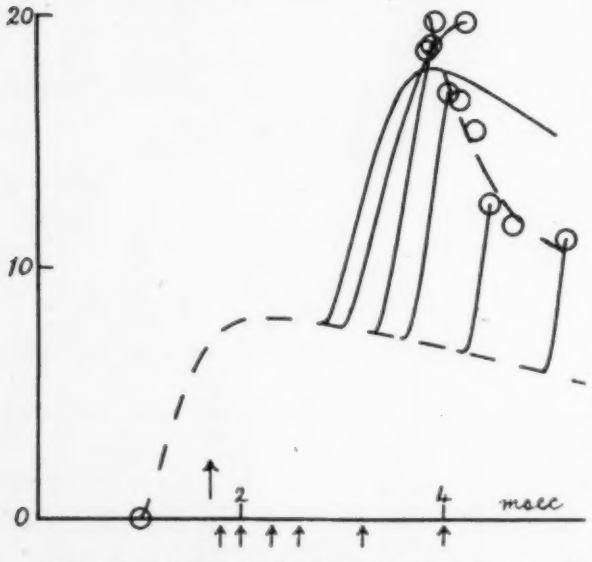

FIG. 15. As in fig. 14, but in a simple spike experiment. E.p.p. curves shown for N_1N_2 intervals marked by arrows. Only spike origins (ringed by circles) shown for other N_1N_2 intervals.

Fig. 15 is plotted similarly to Fig. 14 but for a "simple-spike" experiment. The spike normally appeared to arise when the e.p.p. was zero (cf. Eccles and Kuffler, 1941), but, as in all simple-spike experiments, there is a high e.p.p. threshold for the initiation of spikes by an early N_2—up to 20 per cent of the peak-potential. With N_1N_2 intervals of 2.0 and 2.1 msec. the spike is not initiated until the summed e.p.p. is well above the threshold curve for longer volley intervals (cf. N_1 1.95 msec. N_2 in Fig. 14b), and, at an interval of 1.8 msec., N_2 sets up no spike though the summed e.p.p. is well above the "threshold curve." Thus the simple-spike and double-step experiments closely resemble each other at short volley intervals, but at longer intervals the threshold e.p.p. curve of the former declines more rapidly to its very low normal value.

With the simple-spike experiments it has been suggested that, in normal muscle, impulses are initiated by an exciting mechanism additional to the e.p.p. (Eccles and Kuffler, 1941). A comparison of Fig. 14 and 15 indicates that this additional mechanism has progressively less action as the N_1N_2 interval is shortened, until at the shortest intervals the e.p.p. appears to be

solely responsible for initiating impulses. A similar transition is observed as muscle is progressively curarized (cf. Eccles and Kuffler, 1941).

In Fig. 14b the e.p.p.'s set up by a nerve volley (N) at various times after an antidromic volley (M) are plotted as broken lines. As the MN interval is lengthened, N sets up a progressively larger e.p.p. (the e.p.p. at zero MN interval is shown by the lower broken line), and a spike is initiated when this e.p.p. approximately attains the normal threshold value, *i.e.* there is no raised e.p.p. threshold as with the N_1N_2 series. The MN and N_1N_2 series similarly differ in simple-spike experiments such as Fig. 3b and 4. The e.p.p. set up by N_1 must, therefore, be responsible for the raised e.p.p. threshold for N_2 in Fig. 14 and 15. On the other hand the diminished production of e.p.p. by the testing nerve volley is a result of the muscle's refractory period, for it is similarly observed with both the MN and N_1N_2 series (cf. Fig. 3b, 4 and 5).

Further evidence that the threshold e.p.p. is raised and recovery delayed by e.p.p. acting during the refractory period has already been given in Sections C1 *c*, *d* and *e*. The effect of subparalytic curarization is of particular interest in this respect. Not only is the least spike-interval shortened (Fig. 13c and d, Section C1, d) and the threshold e.p.p. diminished on account of the smaller e.p.p. set up by N_1, but an early N_2 may actually add a larger e.p.p. than it does normally (cf. Fig. 13b at N_1 1.85 msec. N_2 where the added e.p.p. is initially larger, but decays rapidly). This latter effect is exceptional, but with subparalytic curarization the e.p.p. set up by an early N_2 volley is always much less diminished then that set up by N_1. Possibly, by delaying the muscle's recovery, the e.p.p. of N_1 normally diminishes the e.p.p. produced by a given N_2, and this effect becomes smaller as the e.p.p. of N_1 is diminished by curare, thus counteracting to some extent the direct depressant action of curare on the N_2 response. Such an effect is also indicated by the slightly higher recovery curves observed for the e.p.p. in the MN as compared with the N_1N_2 curves of Fig. 5a and b.

3. DISCUSSION

It has been shown above and by Eccles, Katz and Kuffler, (1941b) that, in the true refractory period of the muscle set up by an antidromic volley, there is a diminished polarizability of the muscle at the endplate region, for the neuro-muscular transmitter sets up a diminished e.p.p. E.p.p. acting during the refractory period gives an additional depressant action—the raised e.p.p. threshold for initiating impulses. Hodgkin (1938, p. 107) found that the polarizability of the nerve membrane was diminished to less than half during the spike peak. His results, *e.g.* Fig. 14, also indicate that the threshold local potential for spike initiation is higher in refractory nerve, and this may well have occurred in our antidromic experiments at intervals too short for the nerve volley to test the e.p.p. threshold.

A later paper will describe the repetitive discharge which the large and prolonged e.p.p. sets up in eserinized muscle, and sometimes in normal muscle.

SUMMARY

Two experimental procedures have been used in determining the size and time course of the endplate potential, e.p.p., during and after the muscle spike potential set up by a single nerve volley.

(i) The potential added by a nerve volley on top of the antidromic spike potential set up by direct stimulation.

(ii) The effect of subparalytic curarization.

It is thus shown that in the cat the e.p.p. attains a maximum of about 10 per cent of the peak-potential and has a time course similar to the e.p.p. added by an early second nerve volley. Two opposing factors modify this time course, the first being normally the stronger: (a) the depolarizing action of the neuro-muscular transmitter continues normally for about 6 msec.; (b) during the muscle's refractory period (up to 6 msec.) the e.p.p. decays more rapidly.

In the frog much less e.p.p. outlasts the spike, suggesting that factor a is briefer than in the cat.

In the cat the e.p.p. coexisting with the muscle spike has been shown to have two actions.

i. It prolongs the muscle's refractory period, lengthening by about 0.81 msec. the least spike-interval for a testing nerve volley.

ii. It raises the threshold level of e.p.p. for initiating a spike.

In action *i*, the e.p.p. resembles the effect of a cathodal polarization directly applied during a nerve's refractory period (Blair and Erlanger). Actions *i.* and *ii.* are both larger if the e.p.p. is increased by an early second nerve volley or by eserinization, and smaller if it is diminished by curarization.

The size of the e.p.p. set up by a testing nerve volley is diminished during the refractory period of the muscle.

We wish to express our thanks to Dr. Bernhard Katz for his valuable suggestions and to the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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